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Impact of fluoride in mammals

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Abstract

Fluoride in low concentration is essential for proper mineralization, development and functions of bones and teeth. But toxicity due to excessive ingestion of fluoride is a matter of grave concern, as many countries have been declared endemic for fluorosis In addition to its toxic effects on bones and teeth, it also causes serious effects on liver, kidney, and reproductive organs and also has teratogenic effect Experimental and clinical studies revealed that withdrawal of fluoride, as well as, administration of vitamins such as vitamin C, D, E and calcium has ameliorative effects

(**Keywords** · fluoride/mammals/toxicity /amelioration)

Introduction

Fluoride is an essential trace element that helps in mineralization, development and functions of bones and teeth. Fluoride in very low levels has been found to help in the prevention of dental caries as well as to lower osteoporosis. However, excessive ingestion of fluorine and its compounds found in water cause a crippling disease known as 'fluorosis' because of its profound affinity for calcified tissues. Thus fluoride is an illustrant of Paracelsus medical concept *'All substances are poison; there is*

none, which is not a poison, only the right dose differentiates a poison and a remedy'. Fluoride toxicity is becoming a matter of grave concern, as many countries have been declared endemic for fluorosis. This makes it imperative for scientists to focus on precise toxic effects of fluoride on skeletal and non-skeletal tissues so that effective therapeutic agents can be developed.

Sources of fluoride

Fluoride is never found free in nature but chemically combines to form fluorite, fluorspar, and calcium fluoride in sedimentary rocks and cryolite and sodium aluminium fluoride in igneous rocks. Owing to their origin in certain types of rocks; fluorides in high concentrations are not common constituent of natural surface waters but may occur in detrimental concentrations in ground waters[1] .

Fluorides are extensively used for increasing the fluidity of melts and slags in glass and ceramic industry. Fluorides are also used as insecticides for disinfecting brewery apparatus. as a flux in manufacture of steel, or preserving wood and mucilage, in chemical industries, for water treatment, and other minor uses. Potential sources of fluoride emission include industrial plants concerned with phosphoric acid and superphosphate productions, aluminium smelters, foundries, glass, brick and tile works, plastic and fluorinated hydrocarbon productions and coal burning[2].

Fluoride is recognized as the most effective caries preventive agent[3,4]. Pan American Health Organization[5] has recommended fluoridation of salts. Similarly fluoridated milk has also been reported to reduce caries[6]. Fluoride tablets and topical application of fluoride preparations on tooth surfaces has been recommended by WHO[7]. Sodium fluoride was also used in the treatment of osteoporosis and osteospongiosis[8-9]. Although fluoride appears to be beneficial keeping the above facts in mind, more research is needed in this area to study the effective dose ranges[10].

Incidence of fluorosis

According to the WHO standards, the permissible level of fluoride is 1 ppm. Beyond the permissible level, fluoride is known to cause fluorosis[11] which is a crippling disease and has been reported in developed and developing nations like India, Kenya, China, Algeria, Argentina, Morocco, Senegal, Turkey, Iraq, Iran, Pakistan, Tanzania, Australia, New Zealand, Japan, Thailand, USSR and USA. In USA with up to 8 ppm of fluoride, no other clinical or functional effects except dental fluorosis is anticipated whereas in India skeletal fluorosis has been described with 3 to

4 ppm of fluoride in drinking water[12]. In India, the upper limit has been reduced from 1.5 ppm to 1.0 ppm with a rider 'lesser the fluoride the better' as fluoride is injurious to health[13].

Fluorosis is a serious problem in India affecting 62 million people due to consumption of fluorides contaminated water, which varies from 1 to 48 mg/L[14]. Seventeen out of 32 states have been declared endemic including Andhra Pradesh, Gujarat, Bihar, Madhya Pradesh, Rajasthan, Tamil Nadu and Uttar Pradesh's. Gujarat state has been severely affected where 18 out of 19 districts are prone to fluorosis due to high fluoride content in drinking water. Mehsana, Banaskantha, Amreli, Sabarkantha and Baroda are the most affected districts[16].

Uptake, distribution and excretion

The fluoride gets absorbed through the gastrointestinal tract membrane by diffusion, and because of the low pH, hydrogen fluoride is formed. The absorbed fluoride circulates in the body and then 99% gets retained in hard tissues and remaining is distributed between blood and soft tissues[17]. Amongst soft tissues higher concentration of fluoride occurs in muscle, kidney, liver, milk, bile and urine. The absorbed fluoride is excreted mainly through urine. Human urinary fluoride concentration depends upon and in fact, is nearly equal to the drinking water concentration[3].

Toxicity

The toxicity of fluoride compounds administered orally differed from species to species. The LD_{50} value for male and female rats is 250 mg and 180-mg/kg body weights respectively. Rabbits have an LD_{50} value of 200 mg fluoride/kg body weight. The LD_{50} value of male and female mice is 54.41 and 51.60 mg fluoride/kg body weight. Though the exact mechanism of fluoride toxicity is not clearly known, studies revealed that fluoride kills in acute poisoning by blocking the metabolism of cells either by inhibiting the enzyme or by influencing with the nerve impulse. Impairment of organ function is observed due to cell damage and necrosis. Storage of fluoride in the. skeleton has been blamed as an etiologic factor in the development of renal osteodystrophy[19].

Morphological effects

Oral administration of sodium fluoride caused significant reduction in body weight[20,21]. Verma and Guna Sherlin[22] also reported significant reduction in maternal

body weight gain from day 15 onward of gestation and during lactation. It could be attributed to the lowered feed consumption. The decrease in feed consumption directly after birth could be due to general malnutrition of the mothers. Collins *et al.*[23] observed that feed consumption decreased significantly at 250 ppm-(25.1 mg/L) of sodium fluoride, and body weights of pregnant females reflected feed consumption trends. Al-Hiyasat *et al*[24] also reported significantly reduced maternal body weights and water consumption of sodium fluoride treated rats.

When excess fluoride combines with calcium to form calcium fluoroapatite, teeth become hypoplastic with brown discoloration. This is called as dental fluorosis[10]. People living in endemic fluorotic areas develop dental fluorosis where the developing teeth exhibit mottling, hypoplasia of enamel and premature fall of teeth. As fluoride accumulates in teeth, the calcium is depleted and the dermatan sulphate increases[25]. These sites of dermatan sulphate formation get demineralized, pitted and perforated enhancing cavity formation particularly on the surface of enamel. Chinoy[26] also reported abrupt wear and tear of enamel from dentine in affected tooth.

Effects on nervous system

Accumulation of fluoride in b rain affect enzymes associated with free radical metabolism, energy production, membrane transport and synaptic transmission[27,28]. Malondialdehyde (MDA), the marker of extent of lipid peroxidation, was elevated in the brain of rats treated with 100-ppm fluoride but was without change in rats treated with 30-ppm fluoride. Also levels of total glutathione, reduced glutathione (GSH) and ascorbic acid were elevated only in 30 ppm fluoride-treated rats while these levels were decreased in 100 ppm fluoride-treated rats. On the other hand, the activities of glutathione peroxidase and glutathione-S-transferase were elevated significantly in both 30 ppm and 100-ppm fluoride-treated rats and greater elevation occurred at 30 ppm. These results suggest that fluoride enhances oxidative stress in brain disturbing the antioxidant defense of rats. Increased oxidative stress could be mediating factors in the pathogenesis of fluoride-toxicity in the brain[29]. Long *et al.*[30] reported decrease in the number of nicotinic acetylcholine receptors in the brain of rats exposed to 100 ppm of fluoride, which may be an important factor in the mechanism of brain dysfunction in the disorder. Shashi[31] reported that there was a direct action of fluoride upon the nerve tissue, which was responsible for central nervous system problems such as tremors, seizures and paralysis indicating brain dysfunction in 20 and 50 mg of sodium fluoride in rabbits.

Fluorides are known to suppress the locomotor behavior[20,32]. Reddy *et al*[33]. reported that neuromuscular pathology in patients with osteofluorosis is one of a

chronic denervation and possibly an outcome of entrapment of spinal nerves but selective loss of myelinated fibers also suggest a coexistent toxic neuronal injury. This is also in conformity with the finding that fluoride exhibits behavioral deficits such as reduced mental work capacity in children (12-13 years of age) who grew up in fluoride endemic areas[34] and in workers who had chronic exposure to industrial fluoride[35] and suggests that fluoride has a potential to produce neurobehavioral impairment by altering the function of a neurotransmitter that is responsible for neurobehavioral activities. People suffer from paralysis and some have developed permanent skeletal deformities and damage to spinal cord[16].

Effects on skeleton

Fluoride has potent effects on bone cell structure, function and strength. These effects are mediated by incorporation of fluoride ions in bone to form fluoroapatite and to increase the osteoblasts activity. A minimum of 100 mg fluoride/ml is essential for osteoblasts stimulation[36]. Vigorita and Suda[37] observed significant changes such as increased osteocytic cellularity and irregular arrangement of osteocytes in bone that are characteristic of fluoride-induced abnormal bone formation and serve as pathological marker. Grynpas[38] reported delay in bone mineral deposition by fluoride. Fluoride administered during gestation gets accumulated in highly mineralized bone and also increases the stability of apatite lattice and decreases the solubility of apatite crystals.

Increased calcium accumulation is one of the initial events of fluoride action on osteoblast[39]. Chavassieux *et al.*[40] reported increase in serum osteocalcin in lambs treated with 3.5 mg sodium fluoride/kg body weight for 120 days with increased osteoblast natality and bone formation rate at tissue level and toxic effect at cellular level. Ishiguro *et al.*[41] reported that distribution of fluoride in cortical bone of human rib being highest in periosteal region, decreasing towards the interior and is rare towards the endosteal surface. The strong stimulus for bone formation by fluoride may thus result in calcium deficiency[41].

Kleerekoper[43] reported the potential of fluoride to increase the skeletal mass and to alter the skeletal architecture. Skeletal fluorosis is well known in spine, pelvic and fore arm and is also found to deform metacarpals and phalangeal osteoporosis in hand of human being[44]. Wang[45] has described skeletal transformation in metabolic skeletal disease such as bone resorption in trabeculae and cortex by osteoclasts accompanied by ossification of osteoclasts. Fluoride stimulates osteoclastic activity and the

parathyroid gland resulting in bone resorption and skeletal transformation[46]. Chen *et al.*[46] reported widening of spaces between trabeculae, osteoporosis and increased osteoid in patients with endemic fluorosis in Guizhou province of China.

Teotia *et al.*[47] and Teotia and Teotia[48] were the first to report that skeletal fluorosis is not only confined to adults but also affects the newborns, infants and children. Young and growing bones are highly vascular, metabolically active and accumulate more fluoride than older bones[49]. High fluoride intake cause osteomalacia and diminished bone strength in rats with renal deficiency.

Soft tissues

In addition to the effect on hard tissues, fluoride also manifests its toxicity on soft tissues such as muscle, collagen, liver, kidney, gastrointestinal tract and reproductive organs.

Chronic fluoride intoxication leads to alterations in structure and function of muscle cells with an increase in serum phosphocreatine kinase which is an index of degeneration of muscle fibers and high permeability of plasma membrane as observed in rabbits treated with 50 mg/kg body weight of sodium fluoride for 30 days[50]. Shashi[51] reported reduction of fibers, vacuolization and necrosis in muscle. The above findings indicate that in fluorotic animals the oxidative metabolism and contractile mechanism of muscle were affected.

The occurrence of zonal necrosis is a common feature of sodium fluoride treatment. The hepatic lobules were hyalinized with loss of cells, cytoplasm vacuolized and the arrangement of hepatic cords disturbed[52]. Chinoy *et al.*[53] reported zonal necrosis and pycnosis of nuclei in liver of rats treated with 10 mg sodium fluoride/kg body weight for 30 days. Chinoy[54] correlated liver damage with significant decrease in serum proteins in fluoride-treated rabbits. Juzyszyn[55] reported impaired detoxification capacity of liver due to exposure of ammonium fluoride. Significant rise in activities of serum transaminases (SGOT and SGPT) during induced fluorosis indicate alterations in liver function, as these enzymes are specific marker[56]. Similar results were also reported in goats[57] and in mice[58]. All these findings support that fluoride affect liver structure and metabolism.

Kidney is the principal organ through which maximum concentration of fluoride is excreted. Any alterations in its structure would affect its function. In mice following administration of 10, 500 and 1000 ppm sodium fluoride, cloudy swelling of kidney tubular cells, marked necrosis and atrophy of glomeruli which affected kidney

function, was observed[59]. Decrease in interstitial space with epithelial cell swelling, hydropic degeneration of proximal and distal tubular segments and bleb formation with fine precipitate in glomerular capillaries were observed on perfusion with 1500 µmole sodium fluoride/liter[60]. Exposures of 135 mg fluoride/kg body weight in male wistar rats caused severe renal injury especially in the tubular region and altered alpha glutathione-S-transferase activity thus serve as an useful marker to study fluoride intoxication[61,62].

Stomach and bowel disorders are major features of intolerance to fluoride intake because the stomach and upper portion of bowels are the major pathways through which the halogens enter the blood stream. Villa *et al.*[63] reported small intestine as the main site of absorption of fluoride. Shashi *et al.*[64] reported erosion, necrosis of mucosal and sub-mucosal layers, diffuse punctuate hemorrhages and disintegration of gastric glands and attributed it to the significant decrease in protein synthesis. Rats administered sodium fluoride during gestation shows glandular hyperplasia of stomach, mucosal cell hyperplasia, inflammation in caecum and colon, intestine and catarrhal enteritis and lymphoid hyperplasia of large intestine[65].

Fuji and Tamura[66] reported dilation of blood vessels and significantly reduced blood flow rate, causing accumulation of the circulating blood in the mucosa of gastrointestinal tract causing redness. Muller *et al.*[67] reported gastric mucosal lesions with acute hemorrhage and blood in the gastric lumen. Patients with osteosclerosis receiving 30 mg sodium fluoride/day for 3-12 months reported vomiting and nausea with petechiae, erosions and erythrema on endoscopy whereas scanning electron microscopy (SEM) studies revealed cracked clay appearance, scanty microvilli, and surface abrasions and desquamated epithelium[68]. The gastrointestinal manifestations are mainly due to the formation of hydrofluoric acid in the gut.

Reproductive toxicity

Kumar and Susheela[69] reported nonfunctional sperms with structural defects in spermatids and spermatozoa of fluoride (10 mg/kg body weight) treated rabbits causing infertility. Acrosomal damage and deflagellation leading to decline in sperm motility were reported[70]; withdrawal of sodium fluoride treatment brought about incomplete recovery whereas administration of ascorbic acid and calcium during withdrawal brought about significant recovery of fluoride-induced effects[75]. Susheela and Jethanandini[71] reported male infertility with abnormality in sperm morphology and testosterone level due to fluoride toxicity. Significant reduction in epididymal weight, altered spermatogenic rate leading to reduction in sperm motility, viability and

fertility rate accompanied by significant reduction in sialic acid, protein contents and alkaline phosphatase activity in epididymis of male mice treated with 10 mg sodium fluoride/kg body weight was reported[21]. However, Fl generation male rats exposed in utero and during lactation at 25, 100, 175 and 250 ppm did not adversely affect the testis structure or spermatogenesis in rats[72].

Decline in uterine weight, levels of DNA and RNA, fertility rates and number of implantation sites in fluoride-treated female mice has been reported[73]. Shashi[74] reported atrophy of follicles with oocyte disintegration and necrosis of cells in the ovary of rabbits during experimental fluorosis.

Maduska *et al.*[75] reported rapid transfer of fluoride across the placenta in ewes. However, Ream *et al.*[76] reported that the amount of fluoride crossing the placenta is insufficient to produce morphological changes in bones of weanling rats born to dams given 150 ppm of fluoride. Collins *et al.*[23] reported significant decrease in mean number of implants per litter, however, significant increases in number of fetuses with three or more skeletal variations were observed in sperm positive female rats given 250 ppm (25. 1 mg/kg body weight) sodium fluoride daily throughout gestation. Heindel *et al.*[77] reported that Sprague-Dawley derived rats administered with 300 ppm of sodium fluoride from day 6 to 15 of gestation caused decreased maternal body weight gain and decreased water consumption. However, it did not significantly affect the frequency of post-implantation loss; mean fetal body weight per litter or external, visceral or skeletal malformations. Al-Hiyasat *et al.*[24] also observed significantly lowered number of viable fetuses and increased number of resorptions in sodium fluoride-treated groups. The effect of sodium fluoride ingestion at 0, 25, 100, 175 and 250 ppm in drinking water were measured in rats throughout three generations[78,79]. No cumulative effects were observed for three generations. Number of corpora lutea, implants, viable fetuses and fetal morphological developments were similar in all groups. Based on these results, Collins *et. al.*[78,79] concluded that sodium fluoride up to 250 ppm did not affect reproduction in rats.

Verma and Guna Sherlin[80] conducted an experiment to evaluate the toxic effects of sodium fluoride on the developing rat embryo-fetuses. Mated female rats received 0, 20, 40 and 80 mg of sodium fluoride/kg body weight/day orally from day 6 to 19 of gestation. Observations were made on day 20 of gestation. No treatment related clinical signs were observed in any treatment group except that 3 out of 10 females were found dead in the highest dose group. Sodium fluoride treatment caused significantly lowered rises in maternal body weight and feed consumption. Significant reductions in number of implantation sites, increased number of resorptions, dead

fetuses, reduction in number of viable fetuses and significant reduction in the weight of fetuses per litter. No-significant rises in external malformations were observed in any of the sodium fluoride-treated dams. Increase in skeletal abnormalities such as presence of 14 ribs, wavy ribs, and dumb bell shaped 5th sternebrae and incomplete skull ossifications as well as visceral abnormalities were observed in fetuses of sodium fluoride-treated dams. However, significant rises in mean per litter skeletal and visceral abnormalities were observed in fetuses of dams administered with 40 and 80 mg of sodium fluoride/kg body weight. Significant increase in post-implantation losses and increased number of resorptions/dead fetuses were observed in 40 and 80 mg of sodium fluoride/kg body weight treated dams[80].

Embryotoxicity might be due to oxidative stress. Increased oxidative stress is one of the mediating factors in pathogenicity of fluoride toxicity in the brain[29] and liver[81]. The fluoride-induced embryotoxicity could be due to oxidative damages. High fluoride levels causes accumulation of large amount of free radicals and peroxides by inhibiting superoxide dismutase and glutathione peroxidase activities causing cell damage in people living in areas endemic to fluorosis[82]. Fluoride causes inhibition of superoxide dismutase, glutathione peroxidase and catalase in the ovary and increased lipid peroxidation causing tissue injury. It mainly causes denaturation of proteins and peroxidation of membrane lipids with increased permeability of cell membrane[83].

Occupational exposure of fluoride causes abnormal menstruation and increases the frequency of miscarriages and pregnancy complications among female workers of fluorine factories[84]. Freni[85] reported decreased birth rates in women exposed to high fluoride concentration in drinking water. Increased number of fine villi covered by a thin syncytiotrophoblast coat and decrease in the activity of oxidizing enzymes primarily cytochrome oxidase has been observed in the placenta of women residing in industrial area. Interaction of fluoride with metals can modify the function of enzymes and thereby influence many metabolic processes during the period of intrauterine life also[86]. Maternal-fetal transport of fluoride across the placenta in mothers exposed to high intake of fluoride during pregnancy and the subsequent transfer of fluoride through breast milk to new borns have been reported[87]. Forresteir *et al.*[88] reported that fluoride passes significantly through the placenta in 5th and 6th month of pregnancy and milk teeth starts to develop in uterus. Fluoride hardens and calcifies the blood vessels, thus blood flow to growing fetus is hampered leading to repeated abortions or still birth[89].

The fluoride content of fetal skeleton and teeth increases with age and with the fluoride intake by mothers. Infants born to such mothers exposed to higher fluoride

content developed skeletal fluorosis. Significant pathological changes in a dose-dependent manner were observed in the fetus born to mothers with mottling of teeth[90]. *Glenn et al.*[91] suggests that fluoride may also exert effects on human fetal growth. The babies of mothers who received fluoride tablets during pregnancy were heavier and longer. Increased accumulation of fluoride in fetal bones could be attributed to the exposure of human organism to high fluoride level[92].

Amelioration

Vitamin D[93] and C[94] treatments significantly lowered fluoride-induced reductions in the body weight and feed consumption as well as rise in incidence of skeletal and visceral abnormalities. Guna Sherlin and Verma[95] have reported amelioration of fluoride-induced hypocalcaemia by vitamins. Partial recovery in serum cations on withdrawal of sodium fluoride treatment has been reported in mice[96]. Withdrawal of sodium fluoride treatment during lactation also caused significant recovery in serum changes in both P and Fl generation rat[22].

The significant recovery in body weight and feed consumption on co-treatment with vitamin C, D, C+D+E along with sodium fluoride were reported[21,32]. Significant mitigation in serum glucose level on co-treatment with vitamin C, D, E and C+D+E is due to significant amelioration in feed consumption[32]. Chinoy and Sharma[21] reported complete recovery from fluoride toxicity in reproductive functions in male mice on co-treatment with vitamin E and D alone and in combination.

Significant recovery on co-treatment with vitamin C, E and C+D+E is attributed to the action of these vitamins as free radical scavengers. Wilde and Yu[97] opined that the toxicity of free radical is greater if fluoride can impair the production of free radical. scavengers such as ascorbic acid and glutathione and this can be prevented by additional supplementation with vitamin C and E. The antidotal effect of vitamin E is by preventing the oxidative damage caused by fluoride, which increases peroxides and free radicals of reactive oxygen species. The protective role of free radical scavenger is by the hydrogen donor ability of alpha-tocopherol. Vitamin E channelizes the conversion of oxidized glutathione (GSSG) to reduced glutathione (GSH), which in turn helps compression of mono- and dehydroascorbic acid to maintain ascorbic add levels. It also has an inhibitory effect on conversion of free or protein bound-SH to -SS- group thus maintaining -SH groups[98]. Meng *et al.*[99] reported reversion in osteofluorosis patients after defluoridation of drinking water. Studies carried out by Gupta *et al.*[100] have revealed that treatments with vitamins C and D as well as calcium produced a significant improvement in skeletal, clinical fluorosis and biochemical

parameters in children drinking water containing 4.5 ppm fluoride. Vitamin D stimulates the intestinal absorption of calcium and phosphate thus raising the plasma calcium and phosphate concentrations. In addition, Vitamin D can also stimulate renal tubular reabsorption of calcium and phosphate[101]. Vitamin D plays a crucial role by maintaining the serum calcium and phosphorus concentration thereby supporting cellular processes[102]. A combination of C+D+E was found to be more efficient in the amelioration and could be due to the synergistic action of all the three vitamins.

The poor nutrition is seemed to be an important cause of endemic osteomalacia in a high fluoride environment and increasing dietary energy, calcium, protein and vitamin C may help in prevention especially in pregnant and nursing women and children[102]. Susheela and Bhatnagar[103] reported reversal of fluoride induced cell injury through elimination of fluoride and consumption of diet rich in essential nutrients and antioxidants. Riordan[104] reported decline in dental fluorosis after changes in supplements and toothpaste regimens.

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Tissue schizontocidal efficacy of a novel anti-malarial compound against *Plasmodium yoelii nigeriensis*

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Abstract

Human malaria is one of the biggest problems for the developing and under developed countries. The spread of multidrug resistant malaria has created greater interest in the field of chemotherapeutics and drug management The assessment of the tissue schizontocidal activity of certain novel and the standard antimalarials were made in swiss albino mice against a drug sensitive strain of *Plasmodium yoelii nigeriensis* Parasites were inoculated into the mice at the rate of $1x10^6$ sporozoite through i/v route The standard drugs used were Chloroquine, Primaquine, Pyrimethamine, Cycloguanil and the novel compounds were CDRI 80/53 and WR 238605 Primaquine, Pyrimethamine, Cycloguanil did show curative effect, whereas Chloroquine was found to be ineffective against sporozoite in mice till the dose of 32mg/kg body weight CDRI 80/53 and WR 238605 used as the novel compound were found to be curative with varying responses The comparative analysis of the standard antimalarial with that of the novel compounds prepared at CDRI and the Walter Reed Army Research Institute USA against the rodent malaria parasite *P yoelii nigeriensis* sporozoite are being discussed here

(**Keywords :** malaria/schizontocidal/ chemotherapy/drugs)

Introduction

Human malaria is one of the greatest danger for mankind because approximately 300-500 million people are annually affected[1,2] with more than 2.7 million global death every year[3,4] out of which one million children die only in sub Saharan Africa [5,6] So far none of the available antimalarial drugs have proved very successful against the malaria, because, for almost every available drug there is a report for the development

of drug resistance[4]. During the course of investigation exercise for new antimalarials in recent past many products have been evaluated with varied degree of success Artimisinin a natural product from plant *Artimisinin anua* and its derivatives have been found to have several advantages over the other antimalarial drugs[7-9] . The failure of the global malaria eradication programme did lead to a loss of interest in malaria for a period of about 25 years from the early 1970s to the late 1990; even then 223 new-antimalarial drug developed during the period 1975-96[10] .With a rapid growing population in region with high malaria transmission, it. has been estimated that in the absence of effective intervention strategies the increasing number of malaria cases will double over the next 20 years[2].

The malaria parasites have developed resistance to most current antimalarials[11,] Artemisinin and its derivatices are the only successful drugs that are apparently effective against severe and multidrug resistant malaria, although, artemisinin resistant murine malaria has been generated and characterized under drug Pressure[13]. Malaria had been sheltered for many years from the dangers of resistance because of the outstanding properties of chloroquine and the slow speed at which resistance developed against this drug[14]. The ultimate resistance against very possible drug and the increasing resistance to currently available drug poses a challenge in the treatment of malaria. Hence, new chemotherapeutic agents are urgently needed to treat this pathogenesis.

CDRI 80/53 (Bulaquine)[21] developed by CDRI and WR 238605 developed by Walter Reed Army Research institute USA, are the two compound specially designed to have antimalarial potentials. Hence, the prophylactic aspects of these compounds are being compared with the other available and potential prophylactic drugs (Primaquine and Pyrimethamine)

Materials and Methods

Animal stock- Swiss albino mice of both sexes, weighing 20-22 gm each, were used in the experiments. The animals were housed in clean cages in controlled environment at the Animal House, CDRI, Lucknow and fed on standard diet. The mice had free access to food and drinking water. Hamsters (either sex)/mosquito (*Anopheles stephensi*) were reared for the *in vivo* parasite maintenance.

Parasite- Chloroquine sensitive (London strain) of the *P. yoelii nigriensis* was used. It was maintained in hamsters and Swiss albino mice through *sporozoite* as well as blood-to-blood passages. Mosquitoes (*Anopheles stephensi*)[15-16] reared at insectory

of the CDRI, were fed on the infected hamster with proper gametocyte count. Their guts were dissected on day +6 after feeding on the infected hamsters mosquitoes to determine the level of infectivity. Sporozoite were isolated between days +10 to +12, purified and suspended in saline solution for i.v. injection into the vertebrate host.

Inoculation- Mice were inoculated with approximately 1 x 10^6 sporozoites in 0.2 ml of the saline per mice through tail vein on day '0'.

Drug Regimen - The drugs were administered through the oral route in about 1ml aqueous solution per mice. All mice received 3 doses of different drugs administered on day '-l' , day '0' and day '+l' in accordance with the routinely practiced norms [15,17] (The day '0' being the day of inoculation of parasite into mice). The standard antimalarials with specific dose regimen used were *Chloroquine* 32mg/kg body weight x3 days; *Primaquine* 48mg/kg x3, 32mg/kg x3, 24mg/kg x3, 16mg/kg x3 and 8mg/kg x3; *Pyrimethamine* 8mg/kg x3, 4mg/kg x3, 2mg/kg x3 and 0.5mg/kg x3; *Cycloguanil* 4mg/kg x3, 2mg/kg x3 and 1mg/kg x3; The test compound CDRI 80/53 at 32mg/kg x3, 16mg/kg x3, 8mg/kg x3; and the *WR* 238605 at 16mg/kg x3, 8mg/kg x3 and 4mg/kg x3 (Table 1). Single shot treatment of the drug was also for the prophylactic evaluation of primaquine and WR 238605 on different days (-3, -2, -1 and on day '0' -6 hr) prior to the inoculation (Table 2).

Microscopy- Thin blood smears were made by cutting the tail vein and taking the blood on a clean 25mm x 75mm glass slides free from grease and scratches on daily basis. The slides were fixed in methyl alcohol and stained with 5% Giemsa (BDH) stain diluted with phosphate buffer at pH 7.2. The slides were observed under light microscope at 100X oil immersion. At least 50 spots were observed to declare any slide as "negative"[17].

Table I– Tissue schizontocidal efficacy of certain antimalarial agents against sporozoite induced *P yoelii nigriensis* (London strain) in swiss mice (Inoculums 1x10^6 sporozoites through i/v route)

Drug (oral)	Dose regimen (mg/kg body wt.)x doses*	No of mice	Patency		Protection (%)
			Days	Mean days ± SD	
Chloroquine	32x3	8	5,6,6,6,7	6 0 ± 0.63	Nil
Control		8	5,5,5,6,6,7,8,8	6.25 ± 1.28	Nil
Primaquine	48x3	8	-	-	100

Table I Contd...

Table 1 Contd

Primaquine	32x3	8	-	-	100
Primaquine	24x3	8	6,6,7,7,8	6 8 ± 0 84	37 5
Primaquine	16x3	8	5,5,5,6,6,7	5 57 ± 0 79	25
Primaquine	8x3	8	5,5,5,6,6,6,7,7	5 58 ± 0 84	Nil
Control	-	8	5,5,5,6,6,6,6,7	5 75 ± 0 71	Nil
Pyrimethamine	8x3	8	-	-	100
Pyrimethamine	4x3	8	-	-	100
Pyrimethamine	2x3	8	-	-	100
Pyrimethamine	0.5x3	8	7,8	7.5 ± 0 71	75
Control	-	8	5,5,5,6,6,6,7,7	5 5 ± 0 55	Nil
Cycloguanil	4x3	8	-	-	100
Cycloguanil	2x3	8	-	-	100
Cycloguanil	1x3	8	-	-	100
CDRI 80/53	32x3	8	7,8	7 5 ±0 71	75
CDRI 80/53	16x3	8	5,6,7,8	6 5 ± 1 29	50
CDRI 80/53	8x3	8	5,5,5,6,6,6,6,7	5 75 ± 0 71	Nil
Control	-	8	5,5,5,5,6,6,6,6	5.5 ± 0 53	Nil
WR 238605	16x3	8	-	-	100
WR 238605	8x3	8	8,9,9	8 67 ± 0.58	62 5
WR 238605	4x3	8	6,6,7,7,7,8	6.83 ± 0 75	25
Control	-	8	5,5,6,6,6,6,6,6	5 75 ± 0.46	Nil

* 3 doses of drug were administered on day '-1', day '0' and day '+1'

Table 2– Tissue schizontocidal efficacy of certain antimalarial compound against sporozoites induced *P yoelii nigriensis* (London strain) in single dose schedule

Drug (oral)	Dose regimen (mg/kg body wt)x doses*	No. of mice	Patency		Protection (%)
			Days	Mean days ± SD	
Primaquine	60x1(day 0 and 6hr)	8	8,8, 9,9	5 8 ± 0.57	50
WR 238605	30x1 (day-3)	8	5,5,5,6,67,8,8	5 5 ± 0.53	Nil
WR 238605	30x1(day-2)	8	-	7.83 ± 0 75	25
WR 238605	30x1(day-1)	8	-	-	100
WR 238605	30x1 (day 0 and-6 hrs)	8	5,6,7,7,8	-	100
Control	-	8	5,5,5,6,6,7	5.37 ± 1.0	Nil

*Route of drug administration· Oral, Inoculums $1x10^6$ sporozoites per mouse.

Results and Discussion

The result for chloroquine treatment (shown under Table 1) indicated that the level of patency was almost equal in control (6.25 ± 1.28) and the chloroquine treated group (6.0 ± 0.63) confirming that the chloroquine does not show any prophylactic efficacy [18,19] against the *P. yoelii* sporozoite at 32 mg/kg x3. This is despite that fact that the chloroquine has greater half-life in blood i.e. 5 days [26], it does not appear to kill early merozoites, infecting RBCs because of paucity of chloroquine in blood due to individual's variation in chloroquine amount in blood plasma[27]. The greater affinity of chloroquine with melanin containing tissues can also play some role here[28] (In addition, individual's immune status may also have some role). Primaquine, In contrast, at 48mg/kg x3 and 32 mg/kg x3 proved 100% protective whereas, its 24 mg/kg x3 and 16 mg/kg x3 doses showed 37.5 and 25 % prophylactic efficacies respectively. 8 mg/kg x3 dose regimen however, did not show any protection. Pyrimethamine, at 2 mg/kg x3 drug dose regimen was protective whereas 0.5 mg/kg x3 drug dose regimen gave 75 % protection. Similarly, Cycloguanil gave 100 % protection at only 1 mg/kg x3 drug dose regimen. This apparently is a successful tissue schizontocidal drug. CDRI 80/53(Bulaquine) a novel antimalarial compound

demonstrated moderate tissue schizontocidal activity at 32 mg/kg x3 drug dose regimen in mice and showed only 75 % protection. This however, has great variation as compared to the other reports based on the results on the prophylactic efficacy of the same drug against primate parasite *P cynomolgi* in rhesus monkey where it has shown 100% protection at 3.16 mg/kg body weight at the same treatment schedule[20] could cure the parasite since the drug also has antirelaps efficacy[21]. The lower doses (16 mg/kg X 3 and 8 mg/kg X 3) of the drug either gave partial protection(50%) or prolonged the patency in mice. WR 238605 was another novel compound with 100% protection at 16 mg/kg x3 doses. Its 8 mg/kg x3 drug dose regimen showed 25% protection and 4 mg/kg x3 drug dose regimen did not show any protection.

In another set of experiment (Table-2) the standard drug, primaquine (that, showed a 100% prophylactic efficacy at 48 mg/kg body weight x3 doses) was treated in single shot of 60 mg/kg body weight on day '0' at -6hrs and compared with the novel drug WR 238605. The result indicated that this prophylactic drug[15,21-23] (primaquine) is only 50% protective at 60 mg/kg body weight while the WR 238605 is 100% protective at only 30 mg/kg body weight in swiss albino mice treated on either day '1' or day '0' at -6hrs. This novel drug gave a partial protection (25%). On the treatment at -2 days and a fair amount of delay in patency i.e., 7.83 ± 0.75 as compared to the control (5.37 ± 1.0). The reason to select a 60 mg/kg b.w of primaquine and 30 mg/kg of WR 238605 was based on our previous experiment (Table-1), where almost half of this dose treated in 3 doses had shown a 100% curative findings. This also led to the conclusion that primaquine is comparatively lesser prophylactic in single shot treatment than the WR 238605. Our observations indicate that the compound WR. 238605 is apparently harmless at 30 mg/kg body weight and the animal did not show any apparent toxic impression even at a little greater dose of the compound. We were not able to develop any resistance among the animals against this drug during our early studies as reported against other drugs[12,13] This might be due to the slower speed of the attainment of the resistance as in the case of chloroquine resistance attainment[14] .The activities of the drug at this level of study promises that we may be able develop in near future a very potential chemotherapeutic agent for the treatment of malaria in man. Apparently WR 238605 does not show any resistance but the possibly of the development of resistance against this drug is not ruled out under that circumstance a resistance reversal is again possible by combination chemotherapy or by the treatment of certain cytokines with the drug[4] . Since this study has been made in very lethal parasite i.e., *P. yoelii nigeriensis* which has a natural tendency to express the resistance against certain drugs to whom it has never been exposed[24-25] .One more interesting coincidence has emerged out of this study is that WR 238605 cures sporozoite infection of *P. yoelii*

perfectly at 30 mg/kg body weight and it is very close to the so far, most successful antimalarial drug against the blood schizonts, the chloroquine, which cures the same parasite (in erythrocytes stage)at the minimum effective dose (MED) of 32 mg/kg body weight[24].

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Prolonged toxicity of sub-lethal dosages of chemical pesticides on the body mass and cocoons of *Aporrectodea caliginosa* (Savigny 1826) (Oligochaeta : Lumbricidae) inhabiting Benghazi, Libya

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Abstract

Laboratory studies were conducted to determine the effects of prolonged toxicity of one dosage of each of six chemical pesticides, which were sprayed in Benghazi agroecosystem to control target pests, on the body mass and cocoons of the earthworm *Aporrectodea caliginosa*, a common inhabitant of Benghazi, Libya. The pesticides tried included cypermethrin (pyrethroid), chlorpyrifos (organophosphate), dicofol (chlorinated hydrocarbon), mancozeb (fungicide) and haloxyfopetotyl (herbicide) The sub-lethal dosages selected to determine the body mass of A *caliginosa* were based on the LD_{50} value of each pesticide, and an approximately one twentieth of LD_{50} value of each pesticide was used to determine its toxicity to cocoons and on the rate of hatching There was an increase in the mean body mass of the control and the pesticide treated worms following 10 weeks of exposure ($F=40$; $p<0.01$). A significant difference existed in the rates of change of body mass ($F=39\ 35$; $p<0.01$) between control and pesticide treated worms and the latter showing significantly higher body mass increment when compared with the former. Variations were observed in the incubation time between the control and the pesticide treated cocoons, which were significant ($F=4.4$; $p<0.01$) Exploratory concentrations of the pesticides were tested for cocoon survival. Chlorpyrifos was the most toxic where no cocoon hatchings were observed even after III days of incubation. This was followed by methomyl and mancozeb with greater than 73% and dicofol and haloxyfopetotyl with 53% hatching failures Only cypermethrin seemed to be the least toxic as approximately 80% cocoons survived and hatchings were normal.

(**Keywords :** *Aporrectodea caliginosa* / earthworm / pesticides / body mass / cocoons)

Introduction

There has been a significant increase aimed at improving the management of earthworms as a resource in agricultural soils. Awareness of soil structural decline encouraged many farmers to adopt various techniques to stimulate more efficient and safer methods of transport of nutrients to plants, and the role of earthworms in this direction cannot be underestimated[1].

The farms of Benghazi (32°10'N, 20°06'E) and its neighborhood in Libya are colonised in large numbers by the earthworm, *Aporrectodea caliginosa* and they constitute more than 80% of the total worms collected[2]. To control insects, plant pathogens, rodents, fungi and weeds, pesticides are widely sprayed in the farms, which adversely affect the beneficial animals including earthworms. Carbamates and organophosphate (OP) insecticides are highly toxic, whereas herbicides and fungicides have moderate to mild toxic impacts on earthworms inhabiting Benghazi agroecosystems[3]. The present study investigates, under laboratory conditions, the effects of prolonged toxicity of sub-lethal dosages of some of the chemical pesticides sprayed in Benghazi farms, on the body mass and cocoons of earthworm, *Aporrectodea caliginosa*

Materials and Methods

The pesticides : Benghazi agroecosystems receive various chemical pesticides, fungicides and herbicides. Consequently representations of these groups were selected for the study. Six chemical pesticides were tried and these included the insecticides cypermethrin (pyrethroid), chlorpyrifos (organophosphate), methomyl (carbamate), dicofol (chlorinated hydrocarbon), the fungicide mancozeb and the herbicide haloxyfopetotyl.

Sampling and rearing of *Aporrectodea caliginosa* : Several farms located at Benina, 15 km south of Benghazi city centre were selected as source of these worms. A. *caliginosa* were sampled from the uncontaminated soils. They were found aggregated on the banks of water canals and on the areas surrounding. water reservoirs. Top soil containing the worms were collected through mild ploughing, and the adult worms were separated from the soil by hand sorting, and brought to the laboratory. In the laboratory, the worms were reared in large plastic pans (41 cm long, 35 cm wide, 20 cm high) containing soil-cowdung mixture. To prepare this mixture, 5% of fine powdered dry cowdung was added and mixed thoroughly with 95% of the oven-dried, powdered fine soil of the habitat of these worms. Water was added to its saturation point. The pans were kept in the dark at 21±2°C and 70±10% relative

humidity, and the worms were introduced in the mixture. The moisture of the soil was measured frequently and adequate quantity of water was added as and when required. Worm rearing continued throughout the experimental period and it served as a stock. Worm behavior, such as surfacing and feeding, and their growth and body mass were continuously monitored to ensure that they were properly acclimatized in the laboratory conditions before subjected to different tests.

Toxicity tests on adult worms and cocoons: Soil was used as the medium for studying the prolonged toxicity of chemical pesticides on the body mass of *A. caliginosa.* Soil and cowdung brought from the habitats of these worms were oven dried (separately) at 55°C, cooled and powdered. Later, they were mixed thoroughly at the rate of 5% cowdung + 95% soil. 300 g of soil-cowdung mixture was transferred into glass vessels (10 cm long, 12 cm wide, 10 cm high). The dosages of the pesticides used were 2, 3, 0.4, 8, 8 and 6 mg/kg of cypermethrin, chlorpyrifos, methomyl, dicofol, mancozeb and haloxyfopetotyl, respectively. These sub-lethal dosages were selected after series of trial experiments where the mortality of these worms was not the goal. 140 ml of the above mentioned dosages of each pesticide was added to and mixed thoroughly with the soil-cowdung mixture of each vessel. Five replicates of each dose of pesticide with five sub-adult worms in each replicate were prepared. Control (5 replicates) contained the worms in water + soil-cowdung mixture. Vessels were checked weekly for 10 weeks to record the body mass of the worms.

To study the impact of sub-lethal dosages of chemical pesticides on the cocoons, one dose of each pesticide was selected The dose selected for each pesticide was approximately one twentieth of its LD_{50} value. The dosages were 50, 50, 25, 62, 125 and 50 ppm of cypermethrin, chlorpyrifos, methomyl, dicofol, mancozeb and haloxyfopetotyl, respectively. Petridishes which were divided into three equal compartments were selected and 10ml of the pesticide dosage was added to each compartment. Water was added to the control. Five freshly laid cocoons of *A. caliginosa* from the stock were introduced into each compartment and the incubation time and hatchings of the worms from the cocoons were noted and recorded daily for III days.

Data were subjected to relevant statistical analyses[4].

Results

Impacts of chemical pesticides on the body mass of *A. caliginosa:* The body mass of *A. caliginosa* kept in the control and in the pesticide treated soil for 10 weeks is presented in Table 1.

Table 1– Body Mass (g) (mean ± S D) of control and pesticide - treated *A. caliginosa* Duration: 10 weeks Total worms exposed· 25 each in control and in each pesticide

Week	Control	Pesticides and concentrations used					
		Cypermethrin (2 mg / kg)	Chlorpyrifos (3 mg / kg)	Methomyl (0 4 mg / kg)	Dicofol (8 mg / kg)	Mancozeb (8 mg / kg)	Haloxyfopetotyl (6 mg / kg)
0	0 34 ± 0.05	0 29 ± 0.03	0.24 ± 0 03	0 27 ± 0.03	0.27 ± 0 03	0.23 ± 0 06	0 31± 0 01
1	0.48 ± 0.07	0 44 ± 0 08	0.23 ± 0 04	0.43 ± 0 06	0.38 ± 0 06	0 20 ± 0 06	0 40 ± 0 01
2	0.50 ± 0.07	0 53 ± 0.07	0.30 ± 0.06	0 50 ± 0.09	0 43 ± 0 08	0 19 ± 0 05	0 50 ± 0.08
3	0 57 ± 0.06	0 62 ± 0 06	0 30 ± 0 04	0.47 ± 0 12	0.46 ± 0.08	0 30 ± 0.06	0 54 ± 0.06
4	0 56 ± 0 08	0 65 ± 0 10	0 37 ± 0 05	0 53 ± 0 12	0 43 ± 0 09	0 35 ± 08	0 57 ± 0 08
5	0 57 ± 0 07	0 60 ± 0 07	0 40 ± 0 03	0 56 ± 0 13	0.50 ± 0.13	0 35 ± 07	0 58 ± 0 13
6	0.64 ± 0 09	0.80 ± 0.12	0 45 ± 0.04	0.59 ± 0.13	0.52 ± 0.10	0.39 ± 10	0 71± 0 24
7	0.66 ± 0.09	0 75 ± 0.08	0.51± 0 04	0 70±0.13	0.62± 0.13	0.52 ·±0 08	0 78 ± 0 25
8	0.65 ± 0 05	0 83 ± 0 11	0 54 0 05	0.73 ± 0 21	0.66 ± 0.11	0 50.±0.10	0 85 ± 0.27
9	0 65 ± 0 06	0 79 ± 0 09	0.55 ± 0 05	0 76 ± 0.14	0.56 ± 0 10	0 52 ± 0 09	0 71± 0 17
10	0 65 ± 0 08	0 86 ± 0 09	0 47 ± 0 13	0 64 ± 0 14	0.62 ± 0 11	0 52 ± 0 14	0 73 ± 0 19

There was an increase in the mean body mass of the control and the pesticide treated worms (except those kept in the mancozeb treated soil) after two weeks of exposure. The increments in the body mass of worms kept in different pesticide treated soils were 83% (cypermethrin), 25% (chlorpyrifos), 85% (methomyl), 59% (dicofol), and 61 % (haloxyfopetotyl). However, there was 17% reduction in body mass of the worms exposed to mancozeb treated soil during this period. In the control, the increase in body mass was 47% during the same period.

From 2-5 weeks, there was a slowing down in the increment of body mass as compared with the previous values (0-2 weeks) in the treated and control worms except those exposed to mancozeb and chlorpyrifos. The percentage increase of body mass of *A. caliginosa* in the control during this period was 14% and those exposed to the soils treated with cypermethrin, methomyl, dicofol and haloxyfopetotyl were 13%, 12%, 16% and 16% respectively. Worms kept in chlorpyrifos treated soil recorded a further increase of 33% as compared with the value of 25% observed for the same compound after 2 weeks of exposure. *A. caliginosa* exposed to mancozeb recovered fast from the negative growth (-17%) observed after 2 weeks of exposure to a higher body mass increment of 84% after 5 weeks.

After 10 weeks, a further body mass increment of 14% as compared with the 5 week value was attained in the control worms. The corresponding increments in the body mass in the pesticide treated worms during this period (5-10 weeks) were 43% (cypermethrin), 18% (chlorpyrifos), 14% (methomyl), 24% (dicofol), 49% (mancozeb) and 26% (haloxyfopetotyl).

In general, worms kept in the pesticide-treated soils recorded a higher body mass increment when compared with the controls. The highest body mass increment during the 10 weeks of exposure was observed in the worm kept in cypermethrin (197%) followed by those kept in haloxyfopetotyl (138%) and in methomyl (137%) treated soils. The body mass increments of the worms exposed to dicofol was 130%, to mancozeb 126% and to chlorpyrifos 96%. The control worms recorded a 91 % increase in their body mass.

The ANOVA test on the body mass of *A. caliginosa* revealed a significant difference ($F=40$; $p<0.01$) between the initial and final (after 10 weeks) body mass of the control and the pesticide treated worms. A significant difference ($F=39.35$; $p<0.01$) was also discernible in the rates of change of body mass between the control and the pesticide treated worms.

Toxicity of pesticides on cocoons : The pesticides, when used at one twentieth concentrations of their LD_{50} values, exerted variable impact on the cocoons of *A. caliginosa.*

Variations were observed in the incubation time between control and pesticide treated cocoons. Cocoons kept in chlorpyrifos treated medium completely failed to hatch and the degeneration of cocoons started after seven days of exposure. The average incubation time for the cocoons kept in control was 45 days, which reduced to 37 and 25 days, for cypermethrin and methomyl respectively. On the other hand, longer incubation periods were discernible for the cocoons kept in mancozeb (62 days), dicofol (67 days) and haloxyfopetotyl (68 days) media.

ANOVA test revealed a significant ($F=4.4$; $p<0.01$) difference in incubation time between control and pesticide treated cocoons. T-tests between the same revealed significant differences ($p<0.01$) in incubation time between control and methomyl, dicofol, mancozeb, haloxyfopetotyl treated cocoons. However such a difference was not discernible between control and cypermethrin ($t=1.86$; $p>0.05$) treated cocoons. Significant differences ($p<0.05$) in incubation time were evident between cocoons treated with 1) mancozeb - dicofol, haloxyfopetotyl, 2) cypermethrin ($p<0.01$) - mancozeb, dicofol, haloxyfopetotyl, and 3) methomyl ($p<0.01$) - dicofol, haloxyfopetotyl. However, the difference in incubation time between dicofol and haloxyfopetotyl treated cocoons was insignificant ($t=0.40$; $p>0.05$).

Table 2 shows the total number of cocoons kept in the control and in different pesticide treated media, the number of cocoons hatched, the total hatchlings from cocoons and the mean hatchling/cocoon in each medium.

13 out of 15 cocoons (87%) kept in the control hatched and a total of 21 worms emerged from them giving a mean number of 1.62 hatchlings / cocoon. This was followed by those kept in cypermethrin medium where 80% cocoons hatched liberating a total of 21 worms, giving a mean of 1.75 hatchlings / cocoon. 67% cocoons (10 out of 15) kept in haloxyfopetotyl hatched and 18 worms emerged from them (1.80 hatchlings / cocoon). Comparatively lower number of cocoons (less than 50%) hatched in methomyl, dicofol and mancozeb media. Only 7 out of 15 cocoons (47%) kept in dicofol hatched, and 11 worms emerged from them giving a mean of 1.57 hatchling / cocoon. Cocoon hatchings were very low in methomyl and mancozeb media where 73% cocoons failed to hatch in both media and the number of hatchlings emerged from the former was 5 and from the latter 7, giving mean of 1.25 hatchlings / cocoon for methomyl and 1.75 for mancozeb.

Table 2– *A. caliginosa* Number of cocoons hatched and the mean hatchling / cocoon in control and in different pesticides

Control and Pesticides	Concentrations of pesticides	Total number of cocoons	Number of cocoons hatched	Number of hatchlings	Mean hatchling / cocoon
Control	---	15	13	21	1.62
Cypermethrin	50 mg / kg	15	12	21	1 75
Chlorpyrifos	50 mg / kg	15	0	0	0.00
Methomyl	25 mg / kg	15	4	5	1.25
Dicofol	62 mg / kg	15	7	11	1 57
Mancozeb	125 mg / kg	15	4	7	1 75
Haloxyfopetotyl	50 mg / kg	15	10	18	1.80

Discussion

A. caliginosa exposed to the sub-lethal dosages of chemical pesticides for a prolonged period of time showed an increase in the body mass when compared with the control worms. With the exception of the worms kept in the mancozeb treated soil, all other pesticide treated worms revealed an initial increase followed by a slowing down and then an exponential increase in their body mass. Worms kept in mancozeb treated soil showed an initial decrease followed by a recovery and a fast increase in their body mass, whereas the control worms showed a steady and uniform increase in body mass throughout the period of observation. The effects of pesticides on cocoons, however, were variable, where most of the pesticides adversely affected the cocoons and their hatchabilities. The incubation period of pesticide treated cocoons also showed wide variations when compared with the control ones. The increase in the body mass of pesticide treated worms over controls could either be due to their increased feeding rates and/or metabolism or due to the accumulation of water in their body tissues to overcome the pesticide poisoning of the environment in which they were kept. Since the sub-lethal dosages of pesticides did not cause any mortality, these worms could recover rapidly from the initial impacts of pesticides and later many were found hyperactive after a certain period of exposure when compared with the control worms.

The sub-lethal dosages of the pyrethroid cypermethrin exerted a positive impact on the body mass of *A. caliginosa* and the effects of this pesticide on cocoons and their hatchabilities were also mild. 80% cocoons kepts in this pesticide medium hatched and a reduction in incubation time as compared with the control was also evident. Many natural and synthetic pyrethroids tested at the recommended dosages to target pests have been proved to be toxic to earthworms and these compounds are characterized by their quick action and knock down. Pyrethroid compounds exert their actions on nerve membranes by modifying the sodium and potassium channels resulting in depolarization by the membrane[7].

A. caliginosa exposed to sub-lethal concentration of organophosphate (OP) insecticide chlorpyrifos treated soil showed an initial slow increase in its body mass, after which a fast growth was discernible during the remaining observational period. However, it exerted an adverse impact on the cocoons and no hatchlings emerged from them even after their exposure to III days. OP compounds although may be highly toxic initially, do not tend to remain in the soil for an extended period of time making them more safer to use compared to chlorinated hydrocarbons as far as the soil fauna is concerned[8]. The author further stated that these compounds intended for

soil use have little lasting effect on earthworm populations although some can cause heavy kills[8]. OP insecticides are generally not very toxic to earthworms[9]. The toxicity of OP compounds to earthworms is mainly through their ability to inhibit acetylcholine esterase (AchE), the enzyme responsible for the analysis of the neurotransmitter acetylcholine (Ach) in the synaptic region of the nervous system, and this is considered a vital process for the normal functioning of the nervous system[7,10,11].

The carbamate insecticide methomyl stimulated a rapid increase in body mass of *A. caliginosa* initially followed by a slow and steady increase of the same after two weeks of exposure with a further sharp increase after 7 weeks. Furthermore, methomyl was found to exert only mild effects on cocoons and approximately 70% cocoon survival was noted. The average incubation time was reduced to 25 days when compared with the 45 days recorded for controls. Sevin, the carbamate pesticide, can have a stimulatory effect rather than an inhibitory effect on the growth and survivability of earthworms when applied at low dosages[12]. However, carbamates sprayed at recommended or at high dosages were reported to be toxic to very high toxic to various species of earthworms[3,6,13,14,15]. Skin swelling and necrotic lesions were observed in *A. caliginosa* exposed to methomyl. Carbamates have an analogous action to that of OP compounds, and they carbamylate the AchE enzyme. However, the enzyme usually recovers more rapidly from carbamates than from OP compounds. Carbamates also block the receptors through which Ach performs its normal function of transmitting the nerve impulses across the synapse[8].

An increasing trend in body mass observed initially in *A. caliginosa* exposed to sub-lethal dosages of dicofol, the chlorinated hydrocarbon, showed tendencies for levelling off of the same towards the end of the experimental period. However, this compound proved to be toxic to cocoons, where 53% cocoons kept in this pesticide medium failed to hatch and the remaining 47% took almost 67 days to hatch. Being a chlorinated hydrocarbon, dicofol might tend to accumulate in considerable quantities in earthworm tissues. Dicofol, if applied in higher dosages, kills the worms through its affinity for the lipoidal membrane sheath of the axons causing repetitive discharge in the waves which throw the worms into tremors and eventually prostate it. The instability of the axon is due to the presence of this compound in the axon membrane altering its permeability to Na^+ and K^+ ions possibly because of the formation of charge-transfer complex between the compound and certain molecules in the membrane[11].

The sub-lethal dosage of the fungicide mancozeb was found relatively toxic to *A. caliginosa* when compared with the other pesticides tried. This compound caused a

reduction in body mass of these worms during the initial exposure period, from which they could recover after three weeks, and a very slow increment in body mass was discernible thereafter, which took a momentum after 7 weeks of exposure. This fungicide, however, proved to be highly toxic to cocoons where 73% cocoons failed to hatch, and the average incubation time for the remaining 27% cocoons was 62 days, a sharp increase when compared with the control incubation time. The toxicity of fungicides to earthworms had been reported earlier[16,17]. Carbamate fungicides are very toxic to earthworms, whereas other fungicidal groups have moderate toxicity. Mancozeb has the ability to inhibit the glucose-6-phosphate of the worm at the start of the glycolytic chain by their NH-C-group becoming free to combine with the SH-group of this enzyme[11].

The herbicide haloxyfopetotyl, when applied at the sub-lethal dosages, caused mild to moderate stimulatory effects on the body mass of *A. caliginosa* which tended to level off towards the end of the experimental period. 67% cocoons kept in this pesticide medium hatched, but 68 days of incubation was required. Toxic effects were not evident on earthworms from herbicide exposure [18]. Very few herbicides are toxic to earthworms[19]. Haloxyfopetotyl owed its toxicity to its ability to work as antifeedant and some herbicides may inhibit the SH-enzymes of the animal and uncouple the oxidative phosphorylation[8].

Our previous study (unpublished data) on the effects of the recommended dosages of these pesticides on target pests, when applied on *A. caliginosa* in Benghazi agroecosystem exerted moderate to high mortality on the adults of this species and a sharp decrease in their numbers and body mass was evident. From the present study, it is apparent that sub-lethal dosages of the same pesticides can exert stimulatory effects on their body mass, even though these compounds prove to be toxic to cocoons. Further studies are in progress to evaluate the effects of sub-lethal dosages of these pesticides on the breeding behavior and cocoon production of the worms, and also on the fate of hatchlings which emerge from the cocoons subjected to pesticide treatments.

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Studies on the anthelmintic activity of the aqueous extract of *Prunus persica* in common poultry worms *Ascaridia galli* and *Heterakis gallinae*

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Abstract

Fresh fruits of *Prunus persica* were purchased from local market and after thorough washing,, the crude extract was prepared with the help of an electric grinder, Suitable concentrations of 6, 8 and 12% were prepared in distilled water When tested *in vitro* all these concentrations caused mortality of *A galli* and *H gallinae* after a maximum exposure of 9 and 8 hours, respectively, The extract caused significant reduction in glucose uptake, glycogen contents, oxygen consumption, acid and alkaline phosphomonoesterase activity and lactic acid production in both the parasites The possible mode of action of *P persica* is discussed

(**Keywords** : *Prunus persica/Ascaridia galli/Heterakis gallinae*/anthelmintic/poultry worms)

Introduction

The present investigations aim at evaluating the efficacy of *Prunus persica* fruit extract against common poultry worms *Ascaridia galli* and *Heterakis gallinae.*

Materials and Methods

Fresh fruits of *P. persica* were obtained from the local market washed properly and cut into pieces. Crude extract was prepared with the help of an electric grinder and it was filtered through a fine muslin cloth and finally transferred to a volumetric flask to prepare 12% stock solution in normal (0.9%) saline. Concentrations of 8 and 6% were prepared by diluting the stock solution with phosphate buffered normal (0.9%) saline. These concentrations were tested *in vitro* for their anthelmintic efficacy.

The parasites were obtained from the common fowl *(Gallus gallus)* slaughtered in local poultry farms. After several washings in normal saline, these were transferred to an incubation medium containing phosphate buffered saline (pH 7.2) to which 1 gm of glucose/100 ml was added. The requisite quantity of the extract was added to the incubation medium to obtain the required concentration and its effect was compared with untreated controls. Worms were incubated at 38°C. Death was assumed to have occurred when no sign of movement was observed even after a long watch.

The glucose uptake studies were made according to Ahmad and Nizami[1]. After incubation, the amount of glucose left in the incubate was estimated by the method of Hultman[2]. Glycogen was estimated in the homogenates (20% w/v) according to Good *et al.*[3] as modified by Montgomery[4]. Rate of oxygen consumption was measured manometrically[5]. Lactic acid production was measured by the method of Baker and Summerson[6]. Acid and alkaline phosphomonoesterase activity was also determined in homogenates according to Bergmeyer[7], whereas cholinesterase activity was measured[8] using acetylcholine as substrate.

Results

A. Effect of *P. persica* fruit extract on the parasites incubated *in vitro*

Effect of the aqueous extract of *P. persica* fruits was examined on the mortality of adult parasites incubated *in vitro*. The extract (12%) caused cent percent mortality in *A. galli* after an exposure for 5 hrs. and in *H. gallinae* after 3 hrs. At 6% of aqueous extract treatment mortality was observed after 9 & 8 hrs, respectively in A. *galli* and *H. gallinae*.

B. Effect of *P. persica* fruit extract on some biochemical activities of the parasites

(i) Glucose uptake : The glucose uptake was reduced by 51 and 48% in A. *galli* and *H. gallinae*, respectively when exposed to 12% fruit extract of *P. persica* (Table 1).

(ii) Glycogen contents : As shown in Table 1, *P. persica* fruit extract reduced glycogen contents by 32 and 40% in A. *galli* and *H. gallinae*, respectively.

(iii) Rate of oxygen consumption: Changes in the rate of oxygen consumption are shown in Table 2. *P. persica* fruit extract (12%) reduced the rate of oxygen consumption by 51 and 61 % in *A. galli* and *H. gallinae*, respectively.

(iv) Lactic acid production : The lactic acid production was enhanced by 71 and 43% in *A. galli* and *H. gallinae,* respectively (Table 2) when exposed to 12% fruit extract of *P. persica.*

(v) Acid phosphomonoesterase activity : As shown in Table 3, *P. persica* fruit extract (12%) reduced the activity of acid phosphomonoesterase by 57 and 48% in A *galli* and *H. gallinae,* respectively.

(vi) Alkaline phosphomonoesterase activity : In the present studies, *P. persica* caused 62 and 49% inhibition in the activity of alkaline phosphomonoesterase in *A. galli* and *H. gallinae,* respectively at 12% concentration (Table 3).

(vii) Cholinesterase activity · As shown in Table 3 cholinesterase activity was diminuted by 49 and 56% in A. *galli* and *H. gallinae,* respectively with 12% P. *persica* fruit extract.

Table 1– Changes in glucose uptake *(mg/g* wet weight) and glycogen contents (% wet wt) in *A galli* and *H gallinae* after *in vitro* incubation with different concentrations of *P persica* fruit extract

		Concentration		
Parasites	Control	6%	8%	12%
Glucose uptake				
A *galli*	5 5±0 17[a]	4 2±0	3 5±0.14	2 7±0.82
		(23 63)	(36.36)	(50.90)
H gallinae	6 2±0 02	4 8±0 14	4.0±0.55-	3 2±0.14
		(22.58)	(35.48)	(48 38)
Glycogen contents				
A. galli	7.3±0.36	6 4±0.17	5 8±0 14	5 0±0 52
		(12 32)	(20.54)	(31.50)
H gallinae	6 7±0.14	5.4±15 39	4 9±0.17	4 0±0 55
		(19 40)	(26.86)	(40 29)

a. Mean ± S.D.

Values in parentheses are percent change of control values

Table 2– Changes in the rate of oxygen consumption (µl/mg wet weight/hour) and lactic acid production (µ mol/gm wet weight) in *A galli* and *H gallinae* exposed to different concentrations of *P persica* fruit extract

Parasites	Control	Concentration 6%	8%	12%
Rate of oxygen consumption				
A galli	5.5±0 17'	4 3±0 14	3.6±0	2.7±0 82
		(21 81)	(34.54)	(50.90)
H gallinae	4.9±0 2	3 7±0 28	2 8±0.12	1.7±0 13
		(24 48)	(42.85)	(61 22)
Lactic acid production				
A galli	4 3±0 1	5 2±0.282	6 5±0.17	7.3±0.14
		(22 06)	(52 58)	(71.36)
H gallinae	6 0±0 14	7 0±0.28	7 8±0 17	8.6±0 22
		(16.66)	(30)	(43.33)

a Mean ± S D

Values in parentheses are percent change of control values.

Table 3– Changes of acid and alkaline phosphomonoesterase (Phosphatase units) and cholinesterase activity (µ moles acetylcholine/hour) in *A galli* and *H gallinae* following *in vitro* incubation with different concentrations of *P persica* fruit extract.

Parasites	Control	6%	Concentration 8%	12%	I_{50}^{a}	r^{b}
Acid phosphomonoesterase						
A. galli	4.7 ± 0.3[c]	3.7 ± 0.1	2.8 ± 0.26	2.0 ± 0.24	9.896	0.9862
		(21.27)	(40.42)	(57.44)		
H gallinae	5 8 ± 0.14	4.3 ± 0 1	3.7 ± 0.1	3.0 ± 0.1	12 430	0 9657
		(25 86)	(36.20)	(48.27)		

Table 3 Contd .

Table 2 Contd

Alkaline phosphomonoesterase						
A gallı	5.3 ± 0 22	4 3 ± 0 1	3.7 ± 0 28	2 0 ± 0 630	9 637	0.6755
		(18 86)	(30 18)	(62 26)		
H gallınae	4 7 ± 0 14	3 5 ± 0 14	2 9 ± 0 122	2 4 ± 0 161	12 262	0 9919
		(25 53)	(38 29)	(48 93)		
Cholinesterase						
A gallı	7 0 ± 0 3	5 2 ± 0 282	4 4 ± 0 31	3 6 ± 0 1	12 353	0 9945
		(25.71)	(37 14)	(48 57)		
H gallınae	6 2 ± 0 17	4 8 ± 0 14	3.6 ± 0 1	2 7 ± 0.24	10 628	0 9863
		(22 58)	(41 93)	(56 45)		

a. Concentration required for 50% ınhıbıtıon

b r = correlatıon coefficıent of th^ actıvıty of control and treated samples

c Mean ± S.D.

Values ın parentheses are percent change of control values

C. Effect of *P. persica* fruit extract on host tissues.

The host tissue, when exposed to 12% concentration of *P. persica* fruit extract, exhibited reduction of 3.81 %, 3.12%, 4.42% and 4.38% in glucose uptake, acid and alkaline phosphomonoesterase and cholinesterase activity, respectively. The mild or negligible changes observed were not significant.

Discussion

Anthelmintic activity of *P. persica* was first reported by Jawahar *et al.*[9] They however, reported a mild effect of this extract on *A. galli* since they used a lower (1 %) concentration. In the present studies involving *ın vitro* experiments *P. persica* fruit extract caused cent percent mortality both in *A galli* and *H. gallinae* after a maximum exposure of nine hours at all concentrations used. A significant reduction in glucose uptake and glycogen contents (Table 1) in both *A. galli* and *H. gallinae,* observed in present investigations indicate the interference of *P. persica* in carbohydrate

metabolism of the parasites. This is further supported by a decrease in the amount of oxygen consumption associated with concomitant rise (Table 2) in the lactic acid level of the parasites as observed in the present studies. Saz[10] has reported that the inhibition of carbohydrate absorption in helminth parasites living in an environment of relatively low oxygen tension is usually disastrous as they entirely depend on their carbohydrate metabolism. The results of the present studies support these findings since 100% mortality occurred, after 9, 7, 5 hours in *A. galli* and 8, 6 and 3 hours in *H. gallinae* when exposed to 6, 8 and 12% concentrations of P. *persica* extract respectively.

The activity of acid phosphomonoesterase, which is reported[11] to play an important role in the carbohydrate metabolism of the nematode parasites, was also inhibited significantly in both the parasites (Table 3). Some workers[12,13] have demonstrated the implications of acid phosphatase in glycogenolysis. Alkaline phosphomonoesterase was also inhibited considerably at all the concentrations of *P. persica* fruit extract used in present studies (Table 3).

A moderate inhibition of cholinesterase activity with *P. persica.* fruit extract, observed (Table 3) in present investigations is probably an indication of its interference in other metabolic activities of the two parasites also.

P. persica fruit extract is found to be nontoxic to the host, at all the concentrations, as no significant change was observed in the host-tissues incubated with this extract. It may therefore, be concluded that *P. persica* fruit can be used as an effective anthelmintic against *A. galli* and *H gallinae*. It appears that *P. persica* fruit affects mainly through its interference in carbohydrate metabolism of the two parasites.

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Integration of arbuscular mycorrhizal fungi (AMF), neem products, oil cakes and farm yard manure (FYM) for controlling root-knot nematode, *Meloidogyne incognita* infecting ginger

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Abstract

Root-knot nematode, *Meloidogyne incognita* is widely distributed in ginger fields and cause heavy toll of damage to ginger plants resulting in poor rhizome production An ecofriendly approach for nematode management using arbuscular mycorrhizal fungi (AMF), neem product, oilcakes and farm yard manure (FYM) in different combinations were evaluated on ginger plant In the first experiment a field trial was conducted to find out the combined effect of AMF [*Glomus fasciculatum* and *Glomus mosseae* (GF and GM)] and neem leaf (NL) and nimin. Observation revealed that after 150 days of treatment GF+NL, GF+Nimin, GF+GM+NL gave significant results in reducing the disease incidence and in increasing the plant growth characters when applied in various combinations

In the second trial integrated management of *M incognita* was achieved by different combination of AMF(GF), oilcakes (neem and mustard) and FYM In all the treatments tried, GF+Neemcake, GF+Oilcake (neem + mustard) were found highly effective in reducing the root-knot population with a consequent increase in the growth of ginger plant. Eco-friendly management practices are useful for quality production of ginger plant.

(**Keywords** . *Meloidogyne incognita/ arbuscular* mycorrhizal fungi (AMF)/ *Glomus fasciculatum/ Glomus mosseae/* neem leaf / nimin)

Introduction

The pesticides constitute an involuntary and unacceptable threat to food safety and environment. Ginger (*Gingiber officinale* Rose.) is the most important spice grown in India. It is highly infected by several plant parasitic nematodes. Among the nematodes, more severe damage is caused by *Meloidogyne incognita* causing root-knot disease on ginger which is responsible for severe damage to plant growth parameters and poor quality rhizome production have been reported by Nehra[1]; Nehra and Trivedi[2]. In the ginger a deterioration in quality is very crucial as well as in

quantity since the edible part is. disfigured or damaged by the infection making it unsuitable for marketing or eating.

Well planned combination of two or more control practices will be useful to control nematodes than any of the treatment alone. An investigation was undertaken to manage *M. incognita* in soil by oil cakes, hot water treatment, summer ploughing and solarization of ginger fields, as reported by Vadhera *et al* [3]. Denematization of ginger rhizomes at 45°C for three hours & summer ploughing and covering of soil with polythene trap gave significant yield (59.06%) and reduction in nematode population (soil 71.58%) was reported by Vadhera *et al.*[3] Mohanty *et al.*[4] reported that pre-planting application of neem cake (a.i/ha) followed by post planting application of carbofuran (1 kg a.i/ha) 45 days after planting gave the best result in term of suppression of root-knot nematode population, disease intensity and increased yield of ginger.

The present investigation was undertaken to manage root-knot nematode, *M. incognita* on ginger by using AMF, neem products, oil cakes and FYM in an integrated manner.

Materials and Methods

Isolation and identification of indigenous AMF

The indigenous AMF were isolated from local fields. Spores/sporocarps, hyphae and root bits with spores were separated from the field soil with the help of 53, 105, 250 and 710 μm sieves. By preparing diagnostic slides of spores, the spore characteristics and their fungal attachment were studied by Gerdemann and Nicolson[5] and these were very helpful for identification of spores. These spores were used to prepare pure culture of individual fungal spp.

Mass production of fungal inocula

Culturing AM fungi on plants growing in disinfected soil has been the most frequently used technique for increasing the number of propagules reported by Menge[6]. After isolating fungal propagules or spore culture in distilled water, the particular isolate was multiplied and stock cultures in the form of colonized roots were maintained. These roots or spores are used to produce large amount of inoculum or soil based media in pots.

Table 1– Integrated effect of AMF (*G fasciculatum* and *G mosseae*) and neem products (Neem leaf and Nimin) for the management of root-knot nematode *M incognita* infecting ginger

S. No.	treatments	Length (cm)		Fresh wt.(g)		Dry wt.(g)		Ginger Yield (g)		No of galls/gm root	No of eggmasses /gm root	No of Eggs/ Eggmass	% Decrease in Eggmasses
		Shoot	Root	Shoot	Root	Shoot	Root	Fresh	Dried				
1.	GM + NL	58 92	15.00	26.10	5 12	1 50	0 52	25 10	5 01	4 01	6 12	232 40	45 00
2.	GM + Nimin .	57.16	13.76	20.24	4 92	1 38	0.50	22.46	4 51	4 76	6 98	244 10	37 24
3.	GF + NL	71 28	24.12	39.01	9.42	2.39	0 40	33 16	6 59	1 01	3 30	200 00	70 31
4	GF + Nimin	68 48	24 00	39 00	9 10	2.38	0 39	30 46	6 01	1 78	3.40	204.16	69 43
5.	GM + GF + NL	65.16	20.15	35.29	8.00	2.00	0.36	31 01	6.21	1.81	3.46	210 00	68 89
6.	GM+GF+NL+ Nimin	65 01	17.42	34.16	7.01	1 69	0 33	28 72	5 69	2 21	4.76	230 00	57 20
7.	'N' alone	35.12	09.46	12.12	2.42	1.22	0 25	06 66	1.20	9.18	11.12	266.00	
8.	CD at 5%	0.630	0 196	0.242	0.336	0.229	6 220	5.390	5 944	5.878	13 23	0.242	

GM- *Glomus mosseae*, GF- *Glomus fasciculatum*, NL-Neem leaf, N-Nematode (Mean of five replicates)

Plant culture

Rhizomes of ginger were surface sterilized with 0.1 % mercuric chloride for two minutes and washed three times in distilled water. Rhizomes were naturally air dried for 1-2 hrs. and raised in autoclaved soil in 30 cm earthen pots. After germination, at three leaf stage these seedlings were transplanted to 16 cm earthen pots.

Nematode inoculum

Single eggmass of *Meloidogyne incognita* was originally separated from brinjal plant and further multiplied on brinjal roots, grown in autoclaved soil. For different experiments, eggmasses were hand picked with the help of sterilized forceps and placed in smaller sieves (8-9 cm diameter) of 1 mm pore size which were priorly mounted with cross layered tissue paper for hatching. These sieves were placed in petridishes containing distilled water and kept in incubator at 27°C. In the experiments freshly hatched L_2S stage juveniles were inoculated to experimental plants.

Farm yard manure (FYM)

Naturally. available farm yard manure was taken and dried in natural environmental conditions. Five gm/pot of powdered FYM was applied for the treatment.

Oil cakes (deoiled seed cakes)

Dried mustard cake (MC) and neem cake (NC) were powdered and applied to experimental pots at the dose of 5 gm/pot.

Neem products

Neem leaf and nimin were tested against root-knot nematode *M. incognita* infecting ginger. Neem leaf powder was prepared in laboratory by drying and powdering it. Coated nimin (3 gm + 100 gm Urea) was used in the experiment.

Experiment I

In the first trial studies were undertaken for evaluation of the combination of AMF and neem products (neem leaf and nimin) under pot trials. AMF was cultured on onion roots. Each control strategy like AMF (*G. fasciculatum* and G. *mosseae*) and

neem products have their own limitations, so in the present study these were evaluated in combination. Four agents were tried viz. G. *fasciculatum,* G. *mosseae,* neem leaf and nimin.

Following combinations of treatments were applied:

1. GM (100 spores) + NL (2.5 gm) / pot
2. GM (100 spores) + Nimin (3 gm)/ pot
3. GF (100 spore) + Neem leaf (2.5 gm)/ pot
4. GF (100 spores) + Nimin (3gm) / pot
5. GM (50 spore) + GF (50 spore) + NL (2.5 gm) / pot
6. GM (50 spore) + GF (50 spore) + Nimin (3 gm) + NL (2.5 gm) / pot
7. 'N' alone (1000 J_2/pot)

GM: *Glomus mosseae,* GF; *Glomus fasciculatum,* NL; Neem leaf, N: Nematode alone; J_2. Second stage juvenile of *M. incognita.*

All treatments were replicated four times. Neem products were applied to the pot soil 15 days prior to ginger plantation for their proper decomposition. AMF treatment was applied one day before plantation. Inoculation by second stage juveniles was applied to the pots 15 days after the ginger plantation. Proper care was taken throughout the season. After 150 days of inoculation all growth parameters as shoot-root height & weight, rhizome production alongwith the root-knot index, number of galls, eggmasses, eggs/eggmass were recorded. Roots were boiled in acid fuchsin and trypan blue for assessing nematode and mycorrhizal infection. All data were statistically analysed.

Experiment II

In this experiment integrated treatment of various bioagents were used in various combinations.

Applied treatments were as follows:

1. GF (100 spores) + NC (5 gm) / pot

2. GF (100 spores) + MC (5 gm) / pot
3. GF 100 spores + (NC + MC) (2.5 gm + 2.5 gm) / pot
4. GF (100 spores) + FYM (5 gm/pot)
5. GF (100 spores) + FYM (2.5 gm) + NC (2.5 gm) / pot
6. GF (100 spores) + FYM (2.5 gm) + MC (2.5 gm) / pot
7. GF (100 spores) + FYM (2.5 gm) + (NC + MC) (2.5 gm + 2.5 gm)/pot
8. 'N' alone (1000 J_2/pot)

GF, *Glomus fasciculatum,* GM, *Glomus mosseae,* NC, Neem cake, MC, Mustard cake, FYM, Farm Yard Manure, N, Nematode alone, J_2, Second stage larvae/Juveniles of *M. incognita.*

Except AMF, all the treatments were applied to pots before two weeks of sowing for proper decomposition. AMF was inoculated alongwith rhizome sowing. After fifteen days of sowing at three leaf stage these plants were inoculated with 1000 freshly hatched juveniles by making holes near the roots and pouring larval suspension into it. All the pots were tagged with full information and proper care was taken during the season. Because of more requirement of water by ginger crops, plants were watered regularly.

After 150 days of inoculation, the effect of different combinations, on the growth parameters of the plants were recorded separately in terms of height, fresh weight and dry weight of root and shoot. Ginger yield was also recorded. The effect on nematode was estimated by counting number of galls, eggmass developed per gm root system and the data were analysed statistically.

Results

Glomus fasciculatum, Glomus mosseae and *Glomus intraradices* were identified from local field soil. Among *Glomus,* G. *fasciculatum* and G. *mosseae* were found to be the most abundant, hence they were used in different experiments.

First trial was conducted to test the efficacy of combined treatment with AMF and neem products. Observation revealed that combined inoculation of all control strategies at their least effective dosage was far better than their single application.

Among all the combinations of treatments, GF + NL was more effective in managing the disease incidence and maximum reduction in nematode reproduction was observed. Maximum shoot length was observed in GF + NL (71.28 cm) followed by GF + Nimin (68.48 cm) and (65.46 cm) in GM + GF + NL treatments respectively. Minimum shoot length (35.12 cm) was recorded in 'N' alone treatment (Table-1). Similar trend was followed by root length, fresh and dry weight of shoot-root which correspondingly increased with the addition of each controlling agent, when compared to nematode alone infected roots where fresh and dry shoot weight (12.12 gm and 1.22gm) were highly reduced. Combination of GF + NL was more effective than other control treatments. Fresh shoot and root weight was recorded as 39.01 gm and 9.42gm in GF + NL combination while in other treatments shoot and root weight was decreased in a chronological order (Table 1). Ginger production was observed at par (33.16 gm) in GF + NL treatment and minimum (6.66 gm) in 'N' alone treated plants.

Significant reduction in nematode population was observed in all the treatments as compared to nematode alone treated pots. Number of galls and eggmasses were the least (1.01 galls and 3.30 eggmasses per gm root) in GF + NL treatment. However, the galls per gm root were ranged from 1.01 to 4.76 and eggmasses per gm root were 3.30 to 6.98 in other treated pots. The galls and eggmasses per gm root were maximum as (9.18 and 11.12) in 'N' alone treated plants. All the data taken were found statistically significant. So in this experiment combinations of arbuscular mycorrhizal fungi and neem leaf proved its effectiveness to nematode population, enhance the growth characters and rhizome production in ginger plant.

In the second experiment over all results showed that when these were treated in different combinations to the pots, gave more biomass production compared to nematode alone inoculated plants and it was also noticed that AMF colonization was not adversely affected by oil cake decomposition. Among the AMF, *Glomus fasciculatum* was selected for the experiment and mustard and neem cakes were evaluated with GF to control *M. incognita* in the investigation.

After the maturation of the plants it was observed that maximum plant growth parameters were given by GF + NC treatments followed by GF + (NC+MC), GF +MC and GF + FYM + (NC + MC) treatments respectively.

Maximum shoot length (60.46 cm), root length (29.78 cm), fresh shoot-root weight (35.78 gm and 10.39 gm) and rhizome production (28.78 gm) were recorded from combination of GF + NC. In some treatment maximum reduction in root-knot index (galls 0.82/gm root, eggmasses = 1.80/gm root) was observed. So this treatments showed its better effectiveness as compared to other treatments.

Maximum number of galls and eggmasses were developed in untreated plants infected with *M. incognita*. Significant reduction in nematode population was observed in all the treatments. Among all the combination treatments, GF + NL was more effective in enhancing the plant growth characters and maximum reduction in nematode reproduction was observed (Table 2). All the data taken were found statistically significant.

Discussion

The integration of different strategies for nematode management is not a new concept. Tyler[7] proposed that the combination of two or more control strategies into an overall management programme is the only sound and sustainable approach for the effective control of root-knot nematode. He stated that, well planned combination of practices will go further towards control of nematodes than any of the treatments alone.

Plant growth parameters were enhanced by the application of different FYM, combinations of oil cakes, AMF and neem products. Neem products have been used to control phytonematodes from many years. 'Nimin' is a triterpene rich material and considered to have some nematicidal properties. From the earlier time 'Nimin' was used as urea coating agent for the control of plant parasitic nematodes. Neem products are efficient to control nematodes because neem is known to be rich in nematode toxic chemicals e.g. azadirachtin, nimbin, nimbidine, remferol etc. It was stated by Akhtar and Alam[8] that 'Nimin' when used as urea coating agent brought about significant reduction in root-knot nematode population and nematode multiplication thereby improved the growth of plants.

The importance of AMF for nutrient uptake is well documented, AMF colonized the root system and may change the post infectional nematode host interaction by altering nematode reproduction and development, reported by Jatala *et al.*[9] Sikora and Sitaramaiah[10] have reported that certain AMF protected the plant from plant parasitic nematodes. Hence the combined effect of AMF and neem products was one of the best opportunities for integrated nematode management.

In the second trial application of GF + Neem cake combination was proved to be the best combination among all treatments with a better plant growth and reduced nematode multiplication. This was in confirmation with Bhattacharya and Goswami [11] who observed an increment in fungal propagules in soil amended with neem cake.

Table 2– Integrated management of root-knot nematode *M incognita* with the combined effect of AMF *(G. fasciculatum)*, oil cakes (mustard 'cake, neem cake) and FYM in ginger'

S. No.	Treatments	Length (cm)		Fresh wt. (g)		Dry wt. (g)		Ginger Yield (g)		No of galls/ gm	No of eggmasses/ gm	No. of Eggs/Egg	% decrease in
		Shoot	Root	Shoot	Root	Shoot	Root	Fresh	Dried	root	root	mass	eggmasses
1.	GF+Nc	68.46	29 78	35 78	10.39	3 56	1.04	28.78	5.52	0 82	1 80	179.00	87.78
2.	GF+Mc	65 00	26 60	29 00	09.18	3.00	0 90	27.00	5.41	2.18	3 10	199 20	79 00
3.	GF+(Nc+Mc)	67.12	27.10	32 40	09.25	3.21	0 94	27.16	5.48	2 01	2.00	192.80	86.42
4.	GF+FYM	52.40	19.72	21 19	06 12	2 20	0 69	20.40	4 00	3.88	5 00	230 00	66 04
5.	GF+FYM+Nc	58.10	20.00	24 18	07.86	2 30	0.70	24.32	4.06	3 12	4.01	222.50	72 76
6.	GF+FYM+Mc	55.22	20 00	24.01	06 65	2 31	0 67	22.10	4 80	3 62	4.78	228.00	67.53
7.	GF+FYM+ (Nc+Mc)	62.60	22 20	25.40	08.01	2 51	0.80	25.90	5.20	3.00	3 64	208.40	75 28
8.	'N' alone	29.40	10.00	13.01	03.16	1 31	0.30	05.71	1.01	8 01	14.72	254.80	-
	CD at 5%	5.36	0.35	2.42	0.62	0.30	2 33	0.63	0.75	0 62	1.54	23.16	

GF - *Glomus fasciculatum*, FYM - Farm Yard Manure. Nc - Neem cake. Me - Mustard cake, N - Nematode (Mean of five replicates)

In the case of integration of oil cakes and fungi, the oil cakes provides nutrients, after decomposition to the fungi and it becomes active in its parasitic stage against nematode reported by Bhattacharya and Goswami [11], while FYM gives consistently higher crop production because of high nitrogen (N_2) and phosphorus (P) content. N_2 and P are essential nutrients for plant growth. Yousif and Badra [12] observed that after decomposition of FYM, same materials (irreversible and reversible) which either permanently damage eggs or delay their hatch are mixed with the test soil.

It may be summarised that INM is a low input approach which calls for a meticulous multidisciplinary analysis at the research levels as well as more sophisticated, skilled management and more information at the farm level than is required for other farming system.

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Biodegradation of crude oil by marine bacteria at Alang (Bhavnagar) sea coast

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Abstract

Four oil degrading isolates designated TVI- TV4 were isolated from oil contaminated sea water at Alang sea coast (60 km from Bhavnagar), known for its ship breaking yard activities Morphological examination of isolates by Gram's reaction suggested isolates TV1 and TV4 to be Gram-positive bacilli, TV2 Gram-positive cocci and TV3 Gram-negative bacilli. Most of the isolates gave negative reaction to IMViC and sugar fermentation tests and positive reaction to catalase test The result of growth (whole cell protein) vs degradation rates (gravimetric-G and spectrofluorometric-SF analysis) of crude oil suggested that isolate TV3 showed maximum growth (0 92 mg/ml) and degradation rate (15 0% G, 15 8 % SF) while TV2 the least growth (0 51 mg/ml) and degradation (10 5% G, 11% SF) on thirty-fourth day of incubation

(**Keywords** . crude oil/ biodegradation / bioremediation)

Introduction

Biodegradation is the metabolic ability of microorganisms to transform or mineralize organic pollutants to less harmful, non-hazardous substances, which are then integrated into natural biogeochemical cycles[1]. The most widely distributed marine organic pollutants are petroleum hydrocarbons/crude oil, spilled into marine environment by tanker accidents, oil spillage, anthropogenic activities, ship breaking yard activities etc. Microbial degradation of crude oil is one of the major routes in the natural decontamination process[2] and microorganisms play a key role in the degradation of petroleum hydrocarbons/crude oil in both terrestrial as well as aquatic environment. One of the recent examples of oil pollution is of Jessica oil spill, which discharged approximately 600 tonnes of diesel and 300 tonnes of bunker fuel at Galapagos Island on 16th January 2001[3] Bioremediation was used to clean up oil-contaminated[4,5,6] sites by spraying N-P fertilizer to accelerate degradative abilities of indigenous microorganisms. Most of the bioremediation techniques currently utilized

are based on enhancement of oil degrading abilities of indigenous microbes by adding nutrients[7].

Alang seacoast, 60 km from Bhavnagar, known for its extensive ship breaking activities, spills large amount of oil on seacoast, proving hazardous to human as well as marine biota Hence, this study aims at developing an effective clean up technology, by exploiting the degradative abilities of microorganisms to remediate oil contaminated sites and keep our seas free of hazardous pollutants.

Materials and Methods

Sampling site : Oil polluted seawater samples were collected from four different ship-breaking plots (1, 2, 3, 4) at Alang seacoast.

Isolation of oil degrading bacteria : For isolation of oil degrading bacteria, crude oil enrichment technique[8] was used 0.1 ml of diluted oil contaminated sea water sample collected from 4 different ship-breaking plots, were streaked on mineral salt medium (MM2) containing $(NH_4)_2SO_4$, 18mM; $FeSO_4.7H_20$, 1µM; $CaCl_2.2H_2O$, 100µM; $MgSO_4 7H_2O$, 1mM and NaCl, 8.5mM in 10mM Na_2HPO_4-K_2HPO_4 buffer and adjusted to pH 7.0. 1.5% agar was added in MM2 before autoclaving. An ethereal solution of crude oil (10% w/v) was uniformly sprayed over the surface of agar plate. The ether immediately vaporized at ambient temperature and thin layer of oil remained on the entire surface. The plates were then incubated at 25°C for sixteen days. The growth of bacteria was compared to control plate without crude oil.

Morphological and biochemical characterization of the isolates : The oil degrading bacteria were characterized by Gram's reaction, IMViC test (indole, methyl red, Vogues Proskauer and citrate), sugar fermentation and catalase tests to facilitate identification of organisms.

Enumeration of heterotrophic and oil degrading bacteria: For enumeration of heterotrophic bacteria R_2A agar medium[9], used specifically for enumeration of bacteria from oligotrophic environment and MM2 medium with crude oil as sole carbon source was used. The oil contaminated seawater samples were diluted to 10^{-3} dilution. 0.1 ml of these diluted samples; collected from 4 different ship-breaking plots were streaked on above media and incubated at 25°C for 4 weeks. The heterotrophic and oil degrading bacteria were enumerated after every 4 days for sixteen days.

Growth vs. degradation of crude oil

Growth : Growth was measured in terms of whole cell protein[10,11]. Cells were harvested by centrifugation (5000g, 10 min) and lysed using 1N NaOH in boiling water bath for 10min. Protein was measured by Lowry's[12] method at the end of 4, 10, 16, 22, 28 and 34 days of incubation

Biodegradation

Preculture preparation . For preculture preparation to be used as inoculum for biodegradation experiment Bushnell-Haas[13] medium (BHM) containing per liter of distilled water NH_4NO_3, 1g; $MgSO_4$ $7H_2O$ 0.2g; $CaCl_2$ $2H_2O$, 0 02g; K_2HPO_4, 1.0g, KH_2PO_4, 1.0g; and $FeCl_3.6H_2O$, 0.05g was used. The medium was supplemented with 5000 mg/L of crude oil. 50ml of medium was dispensed in 250ml Erlenmeyer flasks and inoculated with oil degrading bacteria The flasks were incubated at 25°C for 7 days on a shaker at 175 rpm.

For biodegradation experiment and observing emulsifying activity, 10^6 cells/ml of preculture was used to inoculate BHM supplemented with 5000 mg/L of crude oil and incubated at 25°C for 34 days on a shaker at 175 rpm. The control comprised of uninoculated BHM with 5000 mg/L crude oil Degradative abilities (residual oil) were examined after every 4, 10, 16, 22, 28 and 34 days of incubation by gravimetric (G)[14] and spectrofluorometric (SF)[15] analysis as below ·

Gravimetric analysis : For estimation of oil degradation rates by gravimetric analysis, the above mentioned preculture was used to inoculate BHM supplemented with 5000 mg/L of crude oil as sole carbon source. The control comprised of uninoculated BHM with the same quantity of crude oil. The flasks were incubated at 25°C for 34 days on a shaker at 175 rpm. Residual oil was quantified at above mentioned time intervals by adding 2ml of sulfuric acid reagent, 10ml of petroleum ether and 1 ml of ethyl alcohol to the above flasks and shaken. The mixture was taken in separating funnel. The upper layer of petroleum ether containing residual oil was separated The residual petroleum ether was drained out from the separating funnel, through a filter paper soaked in petroleum ether, in pre weighed glass beaker. The beaker was kept in water bath at 60°C to evaporate petroleum ether. The beaker with residual oil was weighed. The oil degrading activity was evaluated by decrease in weight compared to that of control.

Spectrofluorometeric analysis For estimation of oil degradation rates by spectrofluorometeric analysis, 5ml of n-hexane (HPLC grade) was added in preculture containing flask incubated for 34 days at 25°C with 5000 mg/L of crude oil as sole carbon source and in uninoculated flasks with the same quantity of crude oil that served as control The mixture was transferred in separating funnel and extracted Extraction was carried out twice. The extract was transferred in clean test tube containing 0.4g of anhydrous sodium sulphate. The extract was decanted into another test tube leaving behind sodium sulphate and evaporated to dryness in rotary evaporator under reduced pressure Extract was transferred in volumetric flask and volume was made up to 10ml with n-hexane.

Fluorescence was measured using Perkin Elmer (LS 50) Luminescent spectrophotometer. Crude oil gave maximum fluorescence at 310 nm excitation and 360 nm emission wavelengths Calibration curves were constructed using oil dissolved in n-hexane in concentrations ranging form 1-20 μg/L.

Synchronous excitation/emission spectra were taken of each sample Oil degradation rates were extrapolated from the calibration curve. Since the aromatics in crude oil fluoresce, this technique provides an estimation of the aromatic fraction in the oil

Results and Discussion

Four oil-degrading bacteria designated, as TVI-TV4 have been isolated from oil contaminated seawater samples on mineral agar plates containing crude oil as sole carbon source The isolates grew profusely on the medium containing crude oil compared to control without crude oil.

The morphological and biochemical characterization of the isolates (Table 1) suggested that isolates TV1 and TV4 were Gram-positive bacilli, TV2 was Gram-positive cocci while TV3 was Gram-negative bacilli. All the isolates gave negative reaction to IMViC test and sugar fermentation tests except TVI, which gave a positive reaction to indole test. All the isolates exhibited positive catalase test except isolate TV2, which gave negative reaction.

Enumeration of heterotrophic and oil degrading bacteria (Table 2) isolated from four different plots (1, 2, 3, 4) and incubated up to sixteen days suggested that the count of heterotrophic bacteria increased up to four days. The count of heterotrophic bacteria of plot 1 was 14 x 10^4 cfu/ml, plot 2 was 10 x 104 cfu/ml, plot 3 was 16 x 104

cfu/ml and plot 4 was 11 x 10^4 cfu/ml on fourth day of incubation, thereafter the count remained almost constant during the rest of the incubation period Whereas oil degraders exhibited an increase in number from fourth day onwards up to sixteen days At the sixteenth day of incubation, the count of oil degraders (13-15 x 10^4 cells/ml) corresponded to heterotrophic plate count, suggesting quick adaptation of microorganisms to substrate oil introduced in the environment.

Table 1– Morphological and biochemical characterization of crude oil degrading bacteria

Isolates→ Tests↓	TVI	TV2	TV3	TV4
Gram's reaction	+ bacilli	+ cocci	– bacilli	– bacilli
Indole test	+	–	–	–
Methyl Red	–	–	–	–
Vogues Proskauer	–	–	–	–
Kosser's Citrate	–	–	–	–
Sugar fermentation				
Glucose	–	⊥	⊥	–
Lactose	–	–	–	–
Xylose	–	–	–	–
Maltose	–	⊥	–	–
Mannose	–	–	–	–
Sucrose	–	–	–	–
Catalase	+	–	+	+

+positive result, –negative result, ⊥ only acid production

Table 2– Enumeration of heterotrophic and crude oil degrading bacteria

Plots↓	Heterotrophic bacteria (x 10^4 cfu/ml)			Oil degrading bacteria (x 10^4 cfu/ml)			
Days→	4	8	12	4	8	12	16
1	14	13	13	2	5	12	13
2	10	10	10	1	4	11	12
3	16	15	15	2	5	14	15
4	11	10	10	2	4	9	11

The result of growth vs. degradation rates suggested that both went hand in hand i.e. as growth increased (Table 3); degradation also increased (Table 4) Both started from fourth day onwards and continued up to thirty-four days At thirty-fourth day of incubation, isolate TV3 showed maximum growth (0.92 mg/ml) and degradation (15.0% G, 15.8 % SF) and TV2 the least i.e , growth (0.51 mg/ml) and degradation (10.5% G, 11% SF). The organisms could be arranged in decreasing order of oil degradative abilities as follows

TV3 (15.0 % G, 15.8 % SF) > TVI4 (12% G, 12.6 % SF) > TV1 (11.8% G, 12 2 %SF) >TV2 (10.5% G, 11 % SF)

Table 3– Growth in terms of whole cell protein (mg/ml) of crude oil degrading bacteria

Days→ Isolates↓	4	10	16	22	28	34
TVI	0.11	0 20	0 35	0 50	0 62	0 70
TV2	0.09	0 15	0 28	0 36	0 43	0 51
TV3	0 20	0.35	0 49	0 64	0 79	0 92
TV4	0 16	0.23	0 40	0 58	0 69	0 80

Table 4– Degradation (%) of crude oil by gravimetric (G), spectrofluorometeric (SF) analysis and emulsification activity

Days→	4		10		16		22		28		34		Emulsification activity
Isolates↓	G	SF	G	SF	G	SF	G	SF	G	SF	G	SF	
TV1	0 31	0 4	1 27	1 4	4.0	4 2	6.6	7 0	9 3	9 8	11 8	12 2	++
TV2	0 10	0 2	0 87	1 0	2.8	3 0	5 1	5 4	8 2	8 6	10 5	11 0	+
TV3	0 49	0 6	2 09	2 2	4 45	4 6	8 8	9.0	11 8	12.2	15 0	15 8	+++
TV4	0 15	0 2	1.7	1 8	4 11	4 2	7 2	7.4	10 1	10.6	12.0	12 6	++

+++ good ++ moderate + poor activity

As the growth of the isolates increased (Table 3) even after thirty-fourth day of incubation, they still need to be examined further for degradation rates. The isolates showing higher degradation rate also showed high emulsification activity (Table 4). Bacteria produce extra-cellular emulsifiers that convert large oil droplets to smaller ones (1 μm) in size, increasing the surface area The increase in surface area of emulsified oil droplets corresponds to higher bacterial growth rates, hence high biological degradation rates Thus, smaller the oil droplets, higher the biodegradation rates.

The isolate TV3 showing higher degradation rates can effectively be used for remediation of oil contaminated sites by enhancing their degradative abilities. This would help to develop an effective clean up technology to keep our marine seacoast free of hazardous, carcinogenic pollutants as oil.

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Assessment of different media for mass multiplication of entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin

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Abstract

Growth and sporulation of Pantnagar (PI) and Delhi (DI) isolates of *Beauveria bassiana* were investigated on twenty-one culture media. The media were prepared from broken grains of pulses (Bengal gram, black gram, cowpea, French bean, green gram, lentil, pea and pigeon pea), cereals (maize, pearl millet, rice, sorghum and wheat), oil seeds (groundnut and soybean) and agricultural by-products (chopped *Sesbania* and soybean stems, maize cob and sugarcane bagasse) To compare the performance of the above media, two routinely used fungal growth media (potato dextrose broth or PDB and Sabouraud's dextrose broth or SDB) were prepared in prescribed composition Generally, sporulation by PI was the highest (significant at p=0 01) on pea, very good on SDB > PDB >French bean. fairly good on 13 other media, poor on the two stem media and extremely poor on bagasse and maize cob media However, dry matter production was highest on groundnut and cowpea media, though significantly (p=0 01) different from lentil > SDB > pea > PDB The rice = maize > the two stem + bagasse + maize cob media were the most suitable for dry mass production by PI Spore production by DI was less than PI on all media, though it did best (not significant at p = 0.01) on PDB > SDB > soybean > pea, fairly good on 13 other media and very poor on stem, bagasse and maize cob media The dry mass production by DI was generally higher than PI in some media, the highest being on French bean > pea, soybean, cowpea, SDB, black gram (not significantly different at p=0 01) (0 816 to 1 000 g) However, for PI it was higher on lentil followed by French bean, green gram, soybean, pearl millet, wheat and rice (0 237 to 0 684 g) as compared to DI The grain-based culture media have demonstrated great promise for setting up cottage industry of mass production of the entomopathogenic fungi, particularly the PI

(**Keywords** *Beauveria bassiana* / mass multiplication / grain- based media)

Introduction

The adverse ecological and environmental impact of large-scale usage of synthetic pesticides has attracted worldwide criticisms after publication of *Silent Spring* in

1962 by Rachel Carson. The mass scale environmental awareness had forced the scientists across the world in developing various legal, scientific and technological weapons for combating crop pest problems During the past three decades, the age-old practice of integrated pest management gained importance, with greatest emphasis on biocontrol rationale and less dependence on synthetic chemicals

Extracts of over 10,000 plants particularly those of neem (*Azadirachta indica* A Juss) have been assayed against various pests even at mass scale in Africa and India, and developed into a few commercial formulations[1] Microbial commercial formulations such as *Bacillus thuringiensis* has been used successfully in the control of insect pests in various parts of the world[2,3] In recent years, the Department of Biotechnology of the Government of India has launched various successful programmes for research and development of microbial pesticides[4]. In India, at present, different private companies are producing microbial pesticide formulations using *Bacillus thuringiensis* (Biolep. Dipel, Biobit, Halt etc) and nuclear polyhedrosis virus (Helicide, Sporocide etc) However, some of the limitations in using these pathogens are (i) their mode of action i e the pathogens should be ingested by the pest for getting infection and (ii) rapid build up of resistance in insect population[5] The entomopathogenic fungi can infect the host through contact. Furthermore, the entomofungal pathogens have been reported to cause spectacular epizootics in natural insect population[6,7,8,9,10]. The spores of the entomopathogenic fungi especially those belonging to Deuteromycetes could be formulated and applied like a chemical pesticide[11]. Therefore, the insect pathogenic fungi could be an alternative to those pathogenic bacteria and viruses[5]. The Deuteromycetes fungus, *Beauveria bassiana* (Balsamo) Vuillemin can infect a wide range of insect pests[12] and has a pan-global distribution as a natural soil flora[13]. This fungus is one of the most widely exploited entomopathogens, which is compatible with many pesticides[10]. Earlier investigations also revealed that both Pantnagar (PI) and Delhi (DI) isolates of this fungal species were highly pathogenic to *Helicoverpa armigera* (Hubner) and *Spilosoma obliqua* (Walker)[14,15,16].

This fungus can be grown easily at cottage industry levels for local use at an affordable cost. However, a suitable technique for its mass production should be made available. As the high capacity of spore production is an important physiological characteristic for epizootic potential of entomopathogenic fungi[17], the present research was designed to develop suitable media from available resources for mass multiplication of both isolates of the fungus.

Materials and Methods

Fungal isolates : The native fungal pathogen (PI) was isolated from diseased larva of *S. obliqua* infesting soybean crop at Crop Research Centre, G. B. Pant University of Agriculture and Technology, Pantnagar while Delhi isolate was collected from Indian Agricultural Research Institute, New Delhi. The fungal isolates were maintained on potato dextrose agar (PDA) slants, which were kept in refrigerator at 8°C for further use.

Experimental media : The culture media were prepared from the broken grains of pulses (Bengal gram, black gram, cowpea, French bean, green gram, lentil, pea and pigeon pea), cereals (maize, pearl millet, rice, sorghum and wheat), oilseeds (groundnut and soybean), agricultural by-products (chopped *Sesbania* and soybean stems, maize cob and sugarcane bagasse) and two routinely used fungal growth media (potato dextrose broth or PDB and Sabouraud's dextrose broth or SDB). All the grains were purchased from the local market while agricultural by-products were collected from Pantnagar University campus In the laboratory both PDB (peeled potato : 200 g + dextrose : 20 g + distilled water : 1000 ml) and SDB (dextrose : 40 g + bacto-peptone : 10 g + yeast extract : 1 g + distilled water : 1000 ml) were prepared as per prescribed composition These two media were taken for the comparison of performance of the other culture media For preparing the coarse media, each commodity was washed in tap water and air-dried. The seeds were broken partially while the stems, bagasse and maize cobs were chopped and crushed into very small pieces

For spore production, each coarse medium was soaked separately in 250 ml of distilled water for 24 h Excess water was drained out from the media and air-dried partially and kept in polypropylene bags (7"x9") For grain-based media 100 g and for agricultural by-products media 50 g was used in each replication. For dry matter estimation 100 ml broth (extracts) of each commodity was kept in 250 ml conical flask The broth was prepared by boiling 100 g of each commodity in 300 ml of distilled water for 45 minutes The extract was strained through double layer of muslin cloth and the filtrate was made up to 300 ml by adding distilled water. However, for both sporulation and dry matter estimation 100 ml of PDB and SDB were kept in 250 ml conical flasks. Finally, all the samples were autoclaved at 121^0 C for 15 minutes at 15 psi. The experiments were conducted in three replications.

Inoculation, harvesting and data collection : A loopful of inoculum was introduced in each commodity under aseptic condition and incubated at 25 ± 1^0 C and 85 ± 5 per cent r.h for 20 days. At the end of incubation, the fungus along with the

spent media was spread over paper, air dried at room temperature and blended in electric blender for few minutes and sieved through 15 mm wire mesh Sterilised talcum powder was mixed with PDB and SDB at 2.1 ratio to soak the liquid before blending Thirty mg of each sample was taken for preparing serial dilution for spore counting using a haemocytometer. For dry matter estimation, fungal mat was washed thrice in distilled water and strained through pre-weighed Whatman No 1 filter paper. All the fungal mats along with filter papers were air dried for 12 h and kept in an oven at 80°C for 72 h to record the dry weight of the fungus The data for spore count and dry weight of fungus was subjected to DMRT analysis for comparison[18]

Results and Discussion

Data presented in Table 1 suggests that sporulation by PI was the highest (significant at $p = 0.01$) on pea; very good on SDB > PDB > French bean (1470×10^{10} to 1060×10^{10} conidia), fairly good on 3 other media (625×10^{10} to 935×10^{10} conidia). Unlike grain-based media comparatively poor performance was observed on two stem media (305×10^{10} to 412×10^{10} conidia) and extremely poor on maize cob media and bagasse (1.20×10^{10} to $4\ 96\times10^{10}$ conidia) However, the differences in the performance of majority of the good media were not significant at $p = 0.01$. The dry matter production was higher on groundnut and cowpea media (0.837 g, 0 865 g) though not significantly ($p = 0\ 01$) different from lentil > SDB > pea >PDB (0.684 g to 0.761). The rice = maize > the two stems = bagasse = maize cob media were the most unsuitable for dry mass production by PI (0.119 to 0.237 g) Spore production by DI was less than PI on all media, though it did best (not significant at $p = 0.01$) on PDB > SDB >soybean > pea (828×10^{10} to 1010×10^{10} conidia), fairly good on 13 other media (516×10^{10} to 885×10^{10} conidia), and poor on stems (287×10^{10} and 315×10^{10}) and very poor on bagasse and maize cob media (3.65×10^{10} to 1.15×10^{10} conidia) However, the dry matter production by DI was higher (significant at $p = 0.01$) on groundnut (2.481 g) followed by pea, soybean, cowpea, SDB, black gram and Bengal gram which were not significantly different at $p = 0.01$ (0.816 g to 1 000) while it was fairly good (non-significant at $p = 0\ 01$) on PDB > pigeon pea > French bean> sorghum > pearl millet > lentil > green gram (0 352 to 0.655 g) The seven other media : rice, maize, wheat, bagasse and two stems and maize cob were extremely poor for dry matter production by DI.

Data presented in Table 1 and compared in Table 2 reveals that majority of culture media made of pulses were superior for both growth and sporulation of both isolates Nelson *et al.*[19] indicated higher spore production of *B. bassiana* on rice grain than *B. brongniartii*. Similarly, Batista *et al*[20] reported 8.8 times higher conidia of *B. bassiana* on bean broth than on rice or potato broth. Rabindra[21] recorded higher

conidia production on cowpea grain The present findings were also in agreement with Somasekhar *et al* [22] who recorded better growth and sporulation of both *B. bassiana* and *B brongniartii* on sorghum grain than on agricultural by-products like sugarcane bagasse and cane trash. However, dry matter production by the two isolates reveals that growth of PI was superior (1.79 to 1 29 fold) and inferior (1.16 to 2.96 folds) on groundnut, black gram, soybean, Bengal gram, pea, SDB and pigeon pea media as compared to DI while on rest three media it was almost at par with DI.

Table 1– Spore and dry matter production of two isolates of *B bassiana* on different culture media prepared with food grains and agricultural by-products

Culture medium	Spore production* (conidia/100 g commodity)		Dry matter production* (g)	
	PI	DI	PI	DI
Pea	$147\times10^{10\text{a}}$	$828\times10^{10\text{abcd}}$	0.689^{abc}	1.000^{b}
SDB**	$1360\times10^{10\text{ab}}$	$986\times10^{10\text{ab}}$	0.701^{abc}	0.849^{b}
PDB**	$1150\times10^{10\text{abc}}$	$1010\times10^{10\text{a}}$	0.648^{bcd}	0.655^{cd}
French bean	$1060\times10^{10\text{bcd}}$	$791\times10^{10\text{abcd}}$	0.684^{abc}	0.529^{cde}
Rice	$935\times10^{10\text{cd}}$	$772\times10^{10\text{abcde}}$	0.237^{gh}	0.224^{efg}
Wheat	$920\times10^{10\text{cd}}$	$815\times10^{10\text{abcd}}$	0.325^{fg}	0.169^{fg}
Groundnut	$895\times10^{10\text{cd}}$	$885\times10^{10\text{abcd}}$	0.837^{a}	2.481^{a}
Soybean	$884\times10^{10\text{cd}}$	$918\times10^{10\text{abcd}}$	0.576^{cde}	0.981^{b}
Bengal gram	$880\times10^{10\text{cd}}$	$771\times10^{10\text{abcd}}$	0.513^{cde}	0.816^{bc}
Cowpea	$865\times10^{10\text{cd}}$	$805\times10^{10\text{abcd}}$	0.865^{a}	0.870^{b}
Pearl millet	$854\times10^{10\text{cd}}$	$640\times10^{10\text{de}}$	0.455^{ef}	0.438^{def}
Sorghum	$826\times10^{10\text{cde}}$	$729\times10^{10\text{bcde}}$	0.600^{cde}	0.443^{def}
Pigeon pea	$802\times10^{10\text{cde}}$	$665\times10^{10\text{cde}}$	0.479^{def}	0.555^{cd}
Maize	$788\times10^{10\text{cde}}$	$775\times10^{10\text{abcde}}$	0.221^{gh}	0.170^{fg}

Table 1 Contd..

Table 1 Contd

Green gram	$753x10^{10cde}$	$691x10^{10cde}$	$0\ 610^{bcde}$	$0\ 352^{defg}$
Lentil	$731x10^{10cde}$	$711x10^{10cde}$	$0\ 761^{ab}$	$0\ 437^{def}$
Black gram	$625x10^{10def}$	$518x10^{10ef}$	$0\ 436^{ef}$	$0\ 832^{bc}$
Soybean stem	$412x10^{10ef}$	$287x10^{10i}$	$0\ 124^{h}$	$0\ 094^{g}$
Sesbania stem	$305x10^{10fg}$	$315x10^{10i}$	$0\ 121^{h}$	$0\ 090^{g}$
Sugarcane bagasse	$4\ 96x10^{10g}$	$3\ 65x10^{10g}$	$0\ 125^{h}$	$0\ 160^{fg}$
Maize cob	$1\ 21x10^{10g}$	$1\ 15x10^{10g}$	0.119^{h}	$0\ 036^{g}$

*In a column, means followed by same letter are not statistically different at p=0 01 by DMRT

**Medium used to compare the performance of the other media

Table 2– Comparison of growth potential of Pantnagar and Delhi isolates on different culture media

Culture medium	Comparative efficiency of PI / DI	
	Spore production* (x fold)	Dry matter production* (x fold)
Pea	+1 78	-1.45
SDB**	+1.38	-1.21
PDB**	+1 14	-1 01
French bean	+1 34	+1 29
Rice	+1 21	+1 06
Wheat	+1 13	+1 92
Groundnut	+1 01	-2 96
Soybean	- 1 04	- 1 70
Bengal gram	+1 14	-1 59
Cowpea	+1 07	-1.01
Pearl millet	+1 33	+1 04
Sorghum	+1.13	+1 35

Table 2 Contd

Table 2 Contd

Pigeon pea	+1 21	-1 16
Maize	+1 02	+1 30
Green gram	+1 09	+1 73
Lentil	+1 03	+1 74
Black gram	+1 21	-1 80
Soybean stem	+1 44	+1 32
Sesbania stem	-1 03	+1 34
Sugarcane bagasse	+1 36	-1 28
Maize cob	+1 05	+3 31

*Superiority or inferiority of Pantnagar isolate over Delhi isolate

+ Higher value, - Lower value

Present investigation reveals that majority of grain-based media can be used for mass production of *B bassiana* while the two stem media could show some promise in mass scale production of this fungus However, some limitations of using these agricultural by-products are difficulties in conidial harvesting and comparatively larger labour requirement during preparation of these media Beside this, initially the fungus grows very slowly on the by-products, which may result in suppression of this fungus by common contaminant like fast growing *Aspergillus* spp. on mass scale production. Among the different grain-based media, pea medium could be considered the best as the growth and sporulation of both the isolates were better supported by this medium It is well documented in Table 1 that using 100 g pea medium 10^{12} or more conidia could be produced by both the isolates. In the field, for *B bassiana* the dosage recommended by different workers ranged between 10^{13} to 10^{14} conidia / ha[23]. Therefore, it will be possible to match this dosage recommendation using one kg pea seed. Dust (talc based) or spray (oil in water) formulation can be used for field application. However, from the farmer's point of view the oil-based formulation is easy to apply with the help of a conventional high volume sprayer[24]. Beside this, the production cost of oil-based formulation is comparatively lower than talc based formulation. Spray formulation can be used by mixing with sticker (Hamam toilet soap @ 0.23g / litre of water)[25]; emulsifier (Tween-80 @ 0.02 %) and oil (Sunflower, Groundnut, etc @ 0 18 %)[24]. Addition of oil increases the effectiveness of conidia[26] by preventing desiccation, helping adhesion and spreading of inoculum on the host body[27]. The cost of production of spray formulation of this fungus for one-hectare

land for single application will be : Pea seed @ Rs. 40.00 / Kg = Rs 40.00; Sticker emulsifier and oil = Rs. 20.00; Polypropylene bag @ Re. 1.00 / piece = Rs. 10.00; Labour and Electricity = Rs. 300.00; Others = Rs. 100.00 (Total = Rs. 470.00). The above economics clearly indicates that production cost for fungal spray formulation for single application in one-hectare area is much lower as compared to insecticidal application.

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Solid-state production of ligninolytic enzymes by *Aspergillus niger* using high pressure steam treated bagasse as substrate

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Abstract

Lignin degrading enzymes production by an isolated strain of *Aspergillus niger* utilizing high pressure steam treated bagasse and untreated bagasse supplemented with agriculture residues, employing stationary and solid substrate fermentation were studied In the used media the high pressure steam treated bagasse, which could replace expensive veratryl alcohol, might act not only as nutrient but also as inducer of lignin enzymes The substantial amount of activities for all three major lignin degrading enzymes, i e lignin peroxidase (LiP), manganese peroxidase (MnP) and laccase obtained under some nutritional conditions in solid state fermentation (SSF) were far higher than in stationary fermentation Under optimal conditions of SSF, the maximum activities of the enzymes LiP, MnP and laccase were 0.56, 0 79 and 1 22 U/ml, respectively In addition, supplementation of untreated bagasse with agriculture residues also excreted appreciable amount of ligninolytic enzymes Thus, this would pave the way for production and application of enzymes on large scale

(Key words . *Aspergillus niger*/ bagasse/ ligninolytic enzymes/ solid-state fermentation/ stationary fermentation)

Introduction

Lignin is a three dimensional phenyl propanoid polymer which is considerably resistant to microbial degradation and is second only to the most abundant

regenerating cellulose However, relatively few groups of microorganisms, primarily white rot fungi are the most efficient degraders of lignin[1,2]. Hence much of the research on the lignin degrading enzymes system has centered upon the white rot fungi enzymes especially *Phanerochaete chrysosporium* enzymes[3,4] There is a little information on the ligninolytic enzymes of brown rot fungi, soft rot fungi and actinomycetes[5] However, there are lot of documents on bioremediation and degradation of lignin related compounds by *other* group of fungi [6,7] During recent years great progress has been made in aspects of lignin enzymes and biodegradation Despite the achieved processes, the mechanisms of lignin biodegradation still need to be fully understood and enzyme applications are quite a few Most previous studies have been focused on single organism and carried out in submerged fermentation[8] In addition, synthetic media that require expensive veratryl alcohol are usually utilized in production of ligninolytic enzymes and furthermore, the fermentation period is comparatively long, adding to high cost of production Nowadays, enzyme is produced through solid substrate fermentation which is considered more natural than other techniques as it provides conditions under which microorganisms grow in wild[9] Therefore the present work deals with preliminary studies on production of lignin degrading enzymes by an indigenous strain of *Aspergillus niger* IS4, a deuteromycete, utilizing high pressure steam treated sugarcane bagasse employing solid state fermentation (SSF)

Materials and Methods

Aspergillus niger IS4, isolated from soil samples collected from sites where wastewater of paper and chemical industries were being discharged, was used for the production of lignin degrading enzymes. High pressure steam treated bagasse was prepared by putting the chopped sugarcane bagasse (5-6 cm, containing 15% of the water) into the steam-treatment vessel at 35psi for 30 min[10]. This was used as substrate for production of enzymes in stationary and solid state cultures. In stationary cultures the liquid medium contained 5g of high pressure steam treated bagasse, 0.5g of yeast extract, 1g of $(NH_4)_2SO_4$ and 1 g of Tween-80 per litre: 50 ml of medium was dispensed into a 250 ml of Erlenmeyer flask while solid state culture (SSF) consisted of 5g of high pressure steam treated bagasse and 10 ml of inorganic solution, which contained 1g of Tween 80, 0.5g of $MgSO_4$ and 0.5g of sodium glutamate per litre of solution. The sterilized growth media were inoculated with 2ml of spore suspension (spore density 5×10^6 spores/ml) under aseptic conditions and the flasks for both culture conditions were statically, incubated for 8 days at 30°C in BOD incubator.

Supernatant of the stationary cultures was withdrawn at regular intervals and directly used as the enzyme extract. However, in case of SSF, water (40ml) was added to each flask and were shaken at 100 rev/min for 2h The content of each flask was treated in a stomacher-lab blender for two minutes and then centrifuged at 5000 rev/min for 20 minutes The supernatant was filtered twice The extracts were kept at 4°C and analysed for enzyme activities. In order to ignore the possibility of interference by pigments during enzyme assay in the ultraviolet-zone, we used azure blue, phenol red and catechol as substrate for LiP[11], MnP[12] and laccase[13] enzyme assays, respectively in the visible zone.

Results and Discussion

As shown in Table 1, ligninolytic activities in solid state fermentation (SSF) were far higher than in static fermentation process, which was in accordance to the results reported by earlier workers[14,15]. Further, they reported higher yields of ligninases, especially laccases and MnP in SSF system. The presence of higher MnP and laccase activities in our study agreed with the findings obtained by the earlier researchers that MnP - laccase combination is the most common group of enzymes in white rot fungi. It is also proposed that these fungi either produce non-significant level of LiP or its production requires different nutritional conditions[16]. However, in the present study both kinds of fermentation system showed a considerable amount of lignin peroxidase activity. These results suggest that our indigenous fungal strain *Aspergillus niger* IS4, a deuteromycete, is capable of producing all three major lignin modifying enzymes in appreciable amount in SSF system. This capability of a fungal strain as evident from the literature survey is an unusual phenomenon of fungus catalysing ligninolysis.[17,18] In addition the two kinds of fermentation did not contain veratryl alcohol which showed that the selected high pressure steamed sugarcane bagasse not only provided easily utilizable nutrients (e.g. hydrolyzate of hemicelluloses) for producing enzymes but also possessed inducers to ligninolytic enzyme which could replace expensive veratryl alcohol.

Table 1– Comparison of SSF and Stationary fermentation on production of ligninolytic enzymes

Mode of Fermentation	LiP (U/ml)	MnP (U/ml)	Laccase (U/ml)
Stationary fermentation	0.084	0.186	0 380
Solid state fermentation (SSF)	0.320	0 551	0.938

(Temperature = 35°C, time= 8 days)

To determine the effect of temperature, pH and ratio of solid/liquid on production of lignin enzymes, orthogonal methods of experiments were used[17]. As shown in Table 2, optimum starting pH for LiP, MnP and laccase were 4.5, 5.5 and 5 5, respectively and optimal temperatures for producing the enzymes were 35°C; a lower ratio of solid/liquid was more beneficial to production of the enzymes, Temperature and ratio of solid/liquid had a larger effect on LiP than on MnP and laccase, but the effects of starting pH on LiP, MnP and laccase were opposite to those of the above two factors.

Table 2– Effect of temperature, pH and ratio of solid/liquid on production of ligninolytic enzymes in SSF.

No	Orthogonal design			Enzyme activity (U/ml)		
	T (°C)	pH	Solid liquid (g/ml)	LiP	MnP	laccase
1.	(1)	(1)	(1)	0.220	0 540	0 938
2.	(1)	(1)	(3)	0.117	0 368	0.645
3	(2)	(1)	(1)	0 645	0 565	1 116
4.	(3)	(1)	(2)	0.112	0 478	0 845
5	(1)	(2)	(2)	0.165	0.638	1 085
6.	(2)	(2)	(1)	0 328	0 805	1 265
7	(2)	(2)	(2)	0 265	0 716	1 115
8	(3)	(2)	(3)	0 168	0 525	1 006
9	(1)	(3)	(3)	0 233	0178	0 645
10.	(2)	(3)	(2)	0 228	0 308	0 890
11	(3)	(3)	(1)	0 214	0 315	0 816
12	(3)	(3)	(3)	0 172	0 274	0 612

(T°C) · (1) 30, (2) 35, (3) 40, pH (1) 4.5, (2) 5 5, (3) 6 5, g/ml (1)1/2, (2)1/3 (3) 1/4

Under optimal conditions for the production of ligninolytic enzymes as shown in Fig 1, the secretion of LiP appeared after 72 h of culture growth and reached the maximum after 120 h of incubation, while production of MnP and laccase started earlier and reached the maximum value of production after 96 h of growth. During the maximum period, the maximum activities of LiP, MnP and laccase were, 0 56,0 79 and 1 22 U/ml, respectively

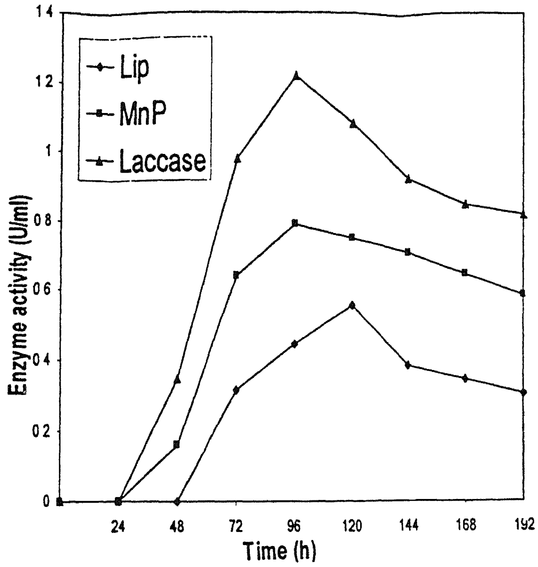

Fig 1– The production profile of LiP, MnP and laccase under optimal condition

Further to minimize the cost of production of enzymes untreated bagasse was also utilized in SSF, however the levels of ligninolytic enzymes activities were very low or almost absent (Table 3). This may be correlated to basic fact that there was no supplementation of fermentation system by inducer of enzymes which may lead to lack of secretion or very low amount of enzymes leading to the inability to degrade the crystallinity of rigid polymeric structure of lignocellulose Therefore untreated bagasse was supplemented with varying concentrations of agriculture processing residues i.e wheat bran, rice bran and pulses waste without disturbing the dry matter content of the culture. Because wheat bran rice bran, and pulses waste contained some starch, proteins and vitamins that were conducive to the growth of fungal strain and enhanced the ligninolytic enzymes production upto 20 to 50 fold. Therefore supplements not only provided easily utilizable nutrients for fungal growth, but possibly also possessed some inducers to lignin enzymes. The growth was more vigorous with increase in proportion of supplements, but enzyme activities were lower

(Table 3) The above phenomenon demonstrated the fact that the growth of *Aspergillus niger* IS4 was inversely related to production of ligninolytic enzymes.

Table 3– Effect of proportions between untreated bagasse and cellulosic supplements on ligninolytic enzymes

Untrated bagasse Cellulosic supplements	Enzyme Activity LiP	(U/ml) MnP	laccase	Biomass (g/g)
Untreated bagasse (UB)	0.010	0 018	0.024	0.21
UB+ wheat bran (19 1)	0 425	0.610	1 210	0 50
UB+ wheat bran (18 2)	0 550	0 656	1.116	0 51
UB+ wheat bran (17 3)	0 365	0.470	0.768	0 63
UB+ wheat bran (16 4)	0.279	0.360	0.750	0.69
UB+ rice bran (19 1)	0 350	0.451	0 820	0 64
UB+ rice bran (18 2)	0.426	0 530	0 956	0.58
UB+ rice bran (17·3)	0 292	0 410	0 845	0 77
UB+ rice bran (16 4)	0.710	0 335	0 720	0 81
UB+ pulses waste (19 1)	0 270	0 340	0 746	0 79
UB+ pulses waste (18 2)	0 310	0 410	0.810	0 72
UB+ pulses waste (17 3)	0 205	0 365	0.682	0.78
UB+ pulses waste (16 4)	0 175	0 275	0.594	0.84

(Temperature = 35°C, time = 8 days)

In conclusion, the SSF technique with high pressure steam treated bagasse as well as untreated bagasse supplemented with defined proportion of agriculture residues as substrate was successfully applied to produce ligninolytic enzymes. This would substantially decrease the cost of production as well as make the overall process more environmentally favourable than static fermentation.

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Repellent property of volatile oil isolated from *Putranjiva roxburghii* against *Trogoderma granarium* associated with stored groundnut seeds

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Abstract

Volatile constituents (essential oils) isolated from leaves of 32 higher plants separately were tested for their repellent activity against *Trogoderma granarium* (collected from stored groundnut seeds) at 0 005, 0 01 and 0 02 ml The oil of *Putranjiva roxburghii* showed 100% repellency at 0 02 ml while others exhibited lower level of repellency The repellency of *Putranjiva* oil was not destroyed by autoclaving, storage (120 days) and temperature treatment (100°C) During *in vivo* investigation, the oil protected the seeds completely from insect (*Trogoderma granarium*) development up to 3 and 6 months separately and was found more efficacious than the synthetic fumigants used The tin containers proved superior to gunny bags for the storage of groundnut seeds Besides, the oil did not show any adverse effect on seed germination, seedling growth, nodulation, general health and morphology of host plant

(**Keywords** insect repellency/ *Trogoderma granarium*/ synthetic fumigants/ *Putranjiva roxburghii*)

Introduction

Several insects attack groundnut and its products in storage. Approximately 6-10% of the groundnut kernels stored in bags are destroyed by insects.[1] *Trogoderma granarium* Everts (Khapra beetle) is one of them.[2] Malathion, lindane and pyrethrum are recommended for control of this insect.[3] The synthetic pesticides have some other adverse effects. Contrary to this, plant based natural pesticides have minimal environmental impact and danger to consumers[4,5]

It is therefore imperative to save groundnut from the infestation of this pest during storage. Plant products, being indigenous resource with insecticidal and insect repellent properties have been in use for over a century, to minimize losses in storage due to insect pests Keeping this in view, screening of volatiles (essential oils) of 32 higher plants was carried out for their insect repellent activity against *Trogoderma*

granarium Everts reared on groundnut seeds under laboratory conditions Besides some *in vitro* and *in vivo* efficacy of the potent oil of *Putranjiva roxburghii* was studied and compared with synthetic fumigants in order to find out the potentiality of oil in preservation of groundnut seeds during storage The phytotoxicity of the oil was also evaluated

Materials and Methods

The stored samples of groundnut seeds (6 to 8 months) were collected from 15 places of different districts of Eastern Uttar Pradesh Four samples of groundnut seeds (200g) from each place of different districts were collected and kept separately in pre-sterilized polyethylene bags after labelling the name of districts and place Different samples of groundnut seeds were observed by hand lens for associated insects.

During screening the leaves of 32 higher plants collected from Gorakhpur and adjoining areas were surface sterilized by dipping in 0 2% mercuric chloride solution for 2 min and then washed repeatedly with sterile, double distilled water The surface sterilized leaves were macerated and hydrodistilled up to 6 h in Clevenger's apparatus for isolation of volatile constituents separately Volatile constituents in the form of essential oil was separated and dehydrated over anhydrous sodium sulphate separately.

0 005, 0 01 and 0 02 ml oil from each plant was soaked in the spongy pieces separately and placed in one of the arms of the 'Y' tube of Olfactometer Water soaked spongy piece was kept in another arm which served as control 20 newly moulted adults of *Trogoderma granarium* of the same age, obtained from the culture maintained in the laboratory, were introduced in the base arm of Olfactometer in 4 batches at an interval of 5 min in order to avoid mutual interference, if any To compensate for possible minor asymmetry in the construction of Olfactometer (since made locally of coming tubes) or in the experimental conditions, test material (oil) and control (water) in the arms were alternated The number of individuals in each arm were counted at the end of test (after 30 min). The experiment was repeated five times for each set of test

Effect of physical factors viz., temperature, autoclaving and storage on insect repellent activity of the potent oil of *Putranjiva roxburghii* was made as following :

The experiment was carried out to find if the insect repellent activity of the oil is thermostable or thermolabile, 4 lots of *Putranjiva* oil, containing 1 ml in air tight specimen tubes were taken and exposed to the different temperature (40, 60, 80 and 100°C) for 60 min in incubator. The specimen tubes containing oil were thus allowed

to cool down to room temperature and the insect repellency of the oil was tested at 0 02 ml against *Trogoderma granarium* by Olfactometer

One ml oil of *Putranjiva* was taken in a air tight specimen tube, autoclaved at 15 lb/sq inch pressure at 120°C for 20 min and the insect repellency was calculated at 0 02 ml by Olfactometer.

Further, the potent oil of *Putranjiva* was stored in air tight specimen tubes at room temperature and its activity against *Trogoderma granarium* was evaluated at regular interval of 15 days at 0 02 ml by Olfactometer

In order to see the effect of *Putranjiva* oil in control of insect development on groundnut seeds during storage, *in vivo* experiment was designed Fresh sample of groundnut seeds was collected in pre-sterilized polyethylene bags. Different amount of oil was used separately with 200 g groundnut seeds kept in pre-sterilized tin containers and gunny bags of 250 ml capacity in order to get the desired concentrations viz., 1000 and 1500 ppm with respect to the volume of the container The sterile cotton swab was soaked with different concentrations of oil, wrapped in sterilized muslin cloth and placed at the bottom of each container to allow complete coverage of stored samples by vapours of the oil Likewise, different lots of groundnut seeds treated with prevalent fumigants (Aluminium phosphide and Ethylene dibromide) were also stored in tin containers and gunny bags. The control sets were prepared by using sterile cotton swab without the oil and the fumigants The containers were closed tightly and kept in grain storage cabinet in the laboratory at room temperature.

The appearance of insects in different containers were observed after 3 and 6 months storage The seeds of treated and control sets after storage were sown in sterilized soil in earthen pots separately Seed germination, seedling growth and nodules were observed at 45 days. General health and morphology of raised plants were also observed.

Results and Discussion

The stored groundnut seeds of different districts exhibited only one population of insect which was identified as *Trogoderma granarium* Everts. It is interesting to note that sample of Basti was badly infested from where maximum insect was recorded.

The essential oil of *Putranjiva roxburghii* exhibited 100% insect repellency at 0.02 ml, while other oils at this amount showed lower level of repellency (Table 1). The oil retained its repellent quality on autoclaving, temperature treatment (up to 100°C) and effective till 120 days (Table 2).

Table 1– Insect repellent actıvıty of essentıal oıls of hıgher plants

Plant specıes (Essential oıl)	Per cent repellency against *Trogodermıa granarıum* at followıng amount (ml)		
	0 005	0 01	0 02
1	2	3	4
Aegle marmelos (L) Correa	30	35	40
Ageratum conyzoıdıs L	40	45	50
A houstonıanum Mıll	40	45	70
Anethum graveolens L	20	30	35
Anısomeles ovata R Br	25	35	40
Artabotrys hexapetalous (Lam) Merr	45	60	70
Azadırachta ındıca A Juss	35	45	50
Caesulıa axillarıs Roxb	35	40	70
Callıstemon lanceolatus DC	35	50	55
Cannabıs satıva L	50	70	75
Cınnamomum tamala Nees and Bbreın	40	45	55
Cıtrus aurantıfolıa Chrıstm	35	45	45
C. medıca var lımonıa (L) Burm F	45	55	75
Eucalyptus cıtrıodora Hook	45	55	60
E globulus (L) Herıt	45	55	60
Eupatorium capıllıfolıum (L) Small	45	55	60
Feronia elephantum Correa	35	40	50
F. lımonıa (L.) Swıngle	40	45	55
Hyptıs suaveolens (L) Poıt	40	50	50
Lantana camera L	55	60	70
L. ındıca Roxb	55	60	70

Table 1 Contd

Table 1 Contd

Mentha arvensis L	25	30	50
M piperata L	25	30	45
M spicata L	25	30	45
Murraya koenigii (L) Spreng	50	65	75
Ocimum adscendens Willd	60	65	75
O basilicum L	45	65	90
O. canum Sims	35	40	45
O sanctum L	35	40	45
Putranjiva roxburghii Wall	70	75	100
Tagetes erecta L.	35	45	50
Thuja occidentalis L	30	40	55

Table 2– Effect of temperature, autoclaving and storage on the insect repellent activity of the oil of *Putranjiva roxburghii*

Physical factors		Per cent insect repellency at 0 02 ml oil
Temperature °C (time of treatment - 60 min)		
	40	100
	60	100
	80	100
	100	100
Autoclaving (15 lb/sq inch pressure at 120°C for 20 min)		100
Storage (in days)	15	100
	30	100
	45	100

Table 2 Contd

Table 1– Insect repellent activity of essential oils of higher plants

Plant species (Essential oil)	Per cent repellency against *Trogoderma granarium* at following amount (ml)		
	0 005	0 01	0 02
1	2	3	4
Aegle marmelos (L) Correa	30	35	40
Ageratum conyzoidis L	40	45	50
A houstonianum Mill	40	45	70
Anethum graveolens L	20	30	35
Anisomeles ovata R Br	25	35	40
Artabotrys hexapetalous (Lam) Merr	45	60	70
Azadirachta indica A Juss	35	45	50
Caesulia axillaris Roxb	35	40	70
Callistemon lanceolatus DC	35	50	55
Cannabis sativa L	50	70	75
Cinnamomum tamala Nees and Bbrem	40	45	55
Citrus aurantifolia Christm	35	45	45
C medica var limonia (L) Burm F	45	55	75
Eucalyptus citriodora Hook	45	55	60
E globulus (L.) Herit	45	55	60
Eupatorium capillifolium (L) Small	45	55	60
Feronia elephantum Correa	35	40	50
F limonia (L.) Swingle	40	45	55
Hyptis suaveolens (L) Poit	40	50	50
Lantana camera L	55	60	70
L. indica Roxb	55	60	70

Table 1 Contd

Table 1 Contd

Mentha arvensis L	25	30	50
M piperata L	25	30	45
M spicata L	25	30	45
Murraya koenigii (L) Spreng	50	65	75
Ocimum adscendens Willd	60	65	75
O basilicum L	45	65	90
O canum Sims	35	40	45
O sanctum L	35	40	45
Putranjiva roxburghii Wall	70	75	100
Tagetes erecta L.	35	45	50
Thuja occidentalis L	30	40	55

Table 2– Effect of temperature, autoclaving and storage on the insect repellent activity of the oil of *Putranjiva roxburghii*

Physical factors		Per cent insect repellency at 0 02 ml oil
Temperature °C (time of treatment - 60 min)		
	40	100
	60	100
	80	100
	100	100
Autoclaving (15 lb/sq inch pressure at 120°C for 20 min)		100
Storage (in days)	15	100
	30	100
	45	100

Table 2 Contd

Table 2 Contd

	60	100
	75	100
	90	100
	105	100
	120	100

Table 3– Appearance of *Trogoderma granarium* in groundnut seeds stored in different containers for 3 and 6 months

Storage period	Control		Treatment					
			Putranjiva oil		Aluminium phosphide		Ethylene dibromide	
	T	G	T	G	T	G	T	G
3 Months	–	–	–	–	–	–	–	–
6 Months	–	+	–	–	–	+	–	+

+	Presence of insect	T	Tin container
–	Absence of insect	G	Gunny bag

Table 4– Nodulation in plants raised from groundnut seeds treated and stored for 3 months and 6 months

Oil/Fumigants	Number of nodules(average)	
	3 months	6 months
Putranjiva oil	53	52
Aluminium phosphide	49	49
Ethylene dibromide	48	46
Control	46	44

As evident from Table 3, that no insect infestation occurred in the groundnut seeds of treatment as well as control sets after three months of storage The insect was

found present in gunny bags of control sets and treatment sets (synthetic fumigant only) after six months of storage. The groundnut seeds stored in tin containers did not exhibit any infestation of insect The oil checked completely the infestation of insect in samples of both the containers stored for six months Table 4, indicates that *Putranjiva* oil treated seeds produced 53 and 52 nodules in 3 and 6 months respectively, while synthetic fumigants treated groundnut seeds and seeds without any treatment showed less number of nodules The oil had no adverse effect on seed germination, seedling growth, general health and morphology of plants raised from the seeds treated with oil These plants were found to be better in comparison to other plants raised from the seeds treated with fumigants The ability of oil and synthetic fumigants to protect groundnut is in following order ·

Oil> Aluminium phosphide> Ethylene dibromide

Previous literature revealed that several pests viz, *Trogoderma granarium, Tenebroides mauritanicus, Rhizopertha dominica* and *Carpophilus dimidiatus*[2], *Caryodon serratus*[6]; *Tribolium castaneum, Oryzaephilus surinamensis* and *Ephestia cautella*[7], *Corcyra cephalonica*[8] and *Plodia interpunctella*[9] have been reported from stored groundnut seeds from time to time, however, in present investigation only one population of insect i e *Trogoderma granarium* was observed in stored groundnut seeds collected from various places of different districts of Eastern Uttar Pradesh

Number of workers reported the repellency of different oils against various insects but repellency of oils against *Trogoderma granarium* is little explored In the present study, the absolute repellency of *Putranjiva* oil against *Trogoderma granarium* is being reported for the first time Earlier the oil of *Putranjiva* has been reported to be toxic against *Helminthosporium oryzae* at a concentration of 500 ppm[10]

It is evident from previous literature that some oils tested in present investigation have also been tested against different storage pests No emergence of *Callosobruchus maculatus* was found on pea seeds when treated with *Citrus* oi[11] *Cinnamomum* oil showed absolute repellency against *Callosobruchus chinensis* at 0 04% conc[12] *Caesulia axillaris* and *Feronia limonia* oils showed 100% repellency at 0.02 ml separately while mixture *(Caesulia + Feronia* 1·1 v/v) exhibited 100% repellency at 0.01 ml[13]. Appreciable pesticidal activity against *Tribolium castaneum* in the oil of *Ocimum basilicum* was found[14]. *Mentha arvensis* oil was found effective against *Sitophilous oryzae*[15]. *Eucalyptus citriodora* oil was found ideally suited against infection by *Rhyzopertha dominica, Oryzaepophilus surinamensis* and *Tribolium castaneum*[16] Powerful acaricidal activities both by direct contact and by inhalation against *Tyrophagus longior* in the oil of *Mentha piperita* and *Eucalyptus*

globulus was found[17] *Azadirachta indica* (neem) oil was found to be a cheap and effective method for controlling of *Trogoderma granarium*, a serious pest of wheat in storage[18]

These oils exhibited absolute repellency against different insects at lower amount but they were not able to repell *Trogoderma granarium* at higher amount indicated the resistance ability of insect In the present study, the oil of *Putranjiva* showed absolute repellency against *Trogoderma granarium* at 0.02 ml indicating its superiority over other oils tested

During recent years, the natural products have been demonstrated to be more effective than several synthetic chemicals used against insect infestation *Cinnamomum* oil was capable of protecting gram seeds from insect infestation up to 12 months[12] Maize grains can be protected up to six months from infestation of *Tribolium castaneum* with the oil of *Caesulia* and *Feronia* at 1000 and 1500 ppm and proved the superiority of oil over synthetic fumigants viz, Aluminium phosphide, Ethylene dibromide[13] The blackgram seeds from infestation of *Callosobruchus chinensis* were protected up to six months with the oil of *Syzygium aromaticum*[19]. Similarily in the present investigation *Putranjiva* oil protected the seeds of groundnut up to six months from insect infestation and was found to be superior than the synthetic fumigants taken into consideration

The present findings suggest that the volatile oil of *Putranjiva roxburghii* due to its strong insect repellent property, persistance of toxicity in storage, no adverse effect on nodulation and general health as well as morphology of the plants, superiority over synthetic fumigants, might be exploited commercially as a preservative for groundnut seeds in storage and to this end trials are being conducted on a large scale

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Performance of certain *capsularis* jute cultivars against jute pests

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Abstract

The field experiments with 16 *capsularis* jute cultivars including JRC-212 and JRC-321 as national varieties and UPC-94 as UP variety have been conducted during *kharif* seasons 2000-01 and 2001-02 to screen out promising cultivars against jute pests, viz. semilooper *(Anomis sabulifera* Guen), stem weevil *(Apion corchori* Marshall) and yellow mite *(Polyphagotarsonemus latus* Banks). The infestation of semilooper, stem weevil and yellow mite varied from 8 64-31 37, 4.17-30 15 and 14 42-37 73 %, respectively The results of the two seasons indicated that the cultivars NDC-8812 and NDC-9801 were significantly superior and most promising against semilooper, stem weevil and yellow mite with an average infestation of 11 07 & 12 62, 8 19 & 5 96 and 14 42 & 18 78 %, respectively On the basis of present data, it is inferred that the *capsularis* jute cultivars NDC-8812 and NDC-9801 are resistant to stem weevil and moderately resistant to semilooper and yellow mite

(Keywords *capsularis* jute cultivars/semilooper/stem weevil/yellow mite)

Introduction

Jute is an important fiber crop extensively cultivated in Northeast and Eastern part of India The *capsularis* jute *(Corchorus capsularis* Linn.) is attacked and damaged by Stem weevil *(Apion corchori* Marshall), semilooper *(Anomis sabulifera* Guen) and yellow mite *(Polyphagotarsonemus latus* Banks) in eastern Uttar Pradesh. The studies on *capsularis* jute germplasm screening against stem weevil have been conducted by Dixit *et al.*[1], Kalita *et al.*[2] and Mohapatra and Patnaik[3]. Das and Pathak[4] have reported that JRC-4444 against semilooper and yellow mite and JRC-7447 against stem weevil are the least susceptible varieties. Recently, we have evaluated advanced olitorius jute cultivars against stem weevil, semilooper, yellow mite and grey weevil (Prasad *et al.*, 2001). However, the evaluation of *capsularis* jute cultivars against jute pests has not been reported. Therefore, an attempt has been made to evaluate the *capsularis* jute cultivars against stem weevil, semilooper and yellow mite under field conditions.

Materials and Methods

The field experiments were conducted in randomized block design with 4 replications during *kharif* seasons 2000-01 and 2001-02 at Crop Research Station, Bahraich, Uttar Pradesh The plot size was 3.0 x 1 5 m^2 with 1 0 m left border all around each plot. The seeds of 13 *capsularis* jute cultivars, viz NDC-9305, NDC-8812, NDC-9101, NDC-9102, NDC-9103, NDC-9801, NDC-9802, NDC-9901, NDC-9902, NDC-9903, NDC-9904, NDC-9905 and NDC-9906 along with the standard national varieties (JRC-212 and JRC-321) and standard Uttar Pradesh variety (UPC-94) were sown at line to line spacing of 30 cm during 1st week of April The crops were raised adopting a standard package of practices except plant protection measures The plant to plant distance was maintained at 5 to 7 cm by thinning after germination of the crop.

The data on different pests, viz. stem weevil, semilooper and yellow mite were recorded at peak infestation during 3rd week of August The infested plants were counted per square meter per plot and presented as per cent plant infestation. For plant infestation, over 3 punctures per plant were considered as stem weevil infested plant, whereas over 3 damaged top leaves per plant have been considered as semilooper and yellow mite infested plant. The infestation of yellow mite was negligible during *kharif* season 2001-02, hence, not reported The data have been statistically analyzed after transforming it by square root transformation formula, $a = \sqrt{p+0.5}$, where a is transformed value and p is per cent infestation.

Results and Discussion

A. Screening of *capsularis* jute cultivars against stem weevil :

The infestation of stem weevil, *A corchori* varied from 4.17 to 30 15 % during *kharif* 2000-02 and its infestation was comparatively low during the year 2001-02 (Table 1) The results clearly indicated that the cultivar NDC-9801 was most promising against stem weevil with 7.75 & 4 17 % infestation during *kharif* seasons 2000-01 & 2001-02, respectively. It was insignificantly followed by the cultivars NDC-8812 with 10.43 & 5.96% and NDC-9901 with 13.85 & 5 98% infestation during the two years, respectively However, the standard, variety JRC-321 was also promising with 12.95 & 6.86% stem weevil infestation during the two years, respectively and did not differ significantly from the cultivars NDC-9801, NDC-8812 and NDC-9901. The average stem weevil infestation in variety JRC-321 and promising cultivars NDC-9801, NDC-8812 and NDC-9901 were 9.90, 5.96, 8.19 and

9.91 %, respectively. Earlier, Dixit *et al.* (1989) have reported that the *capsularıs* jute germplasm with 1-10 % stem weevil infestation are _resıstant, whereas Mohapatra and Patnaik (1995) have rated the *capsularıs* genotypes with 10 5 to 19 3 % stem weevil infestation as moderately resistant Hence, ın the present study, the *capsularıs* cultivars NDC-9801, NDC-88I2 and NDC-9901 may be considered as resıstant against stem weevil.

Table 1– Performance of *capsularıs* jute cultıvars agaınst stem-weevıl durıng *Kharıf* 2000-02

Cultivars	Incidence of stem weevil (%)		Average (%)
	2000-01	2001-02	
NDC-9305	12.45 (3.54)	9 15 (3 06)	10 80
NDC-8812	10.43 (3 19)	5.961 (2 47)	8.19
NDC-9101	13 73 (3 74)	12 011 (3.53)	12 87
NDC-9102	13 76 (3.76)	9 99 (3.22)	11 87
NDC-9103	14.44 (3 83)	14 63 (3 88)	14 53
NDC-9801	7 75 (2 86)	4 1 7 (2 14)	5 96
NDC-9802	23.22 (4.76)	14 65 (3.84)	18 93
NDC-9901	13 85 (3 77)	5 981 (2.53)	9 91
NDC-9902	26 69 (5 19)	10 631 (3 31)	18 66
NDC-9903	25 69 (5 03)	8 64 (2 99)	18 16
NDC-9904	30 14 (5.40)	6 08 (2.50)	18 11
NDC-9905	20.191 (4 52)	7.68 (2.82)	13 93
NDC-9906	22.44 (4 79)	9 50(3 14	15.97
JRC-212	15 28(3 93)	6 38 (2 60	10 83
JRC-321	12 95 (3 65)	6 86 (2 70	9 90
UPC-94	17 77 (4 21)	9 22 (3.11)	13.49
CD (5%)	1.01	0.61	
cv (%)	18 16	15.22	

Figures in parentheses are transformed values

B. Screening of *capsularis* jute cultivars against semilooper and yellow mite.

The infestation of semilooper, *A. sabulifera* varied from 8.64 to 31.37 % during 2000- 02, whereas the infestation of yellow ,mite, *P latus* varied from 14.42 to 37.73 % during *kharif* 2000-01 (Table 2) The results clearly indicated that the cultivars NDC-8812 and NDC-9801 were most promising against semilooper with 8.63 &13.52 and 9.56 & 15 69 % infestation during *kharif* 2000-01 & 2001-02, respectively. However, the standard variety JRC-212 was also promising with 9.58 & 16.50 % semilooper infestation during the two years, respectively and did not differ significantly from the cultivars NDC-8812 & NDC-9801. The average semilooper infestations over the years in variety JRC-212 and the cultivars NDC-8812 and NDC-980I were 13.04, 11.07 and 12.62 %, respectively. Similarly the cultivars NDC-8812, NDC-9305 and NDC-9801 along with standard variety JRC-212 were most promising against yellow mite and did not differ significantly with 14.42, 17,23, 18,78 and 16.48 % infestation during *kharif* season 2000-01, respectively. From the data it is clear that the promising cultivars NDC-8812 and NDC-9801 are not significantly different from the standard variety JRC-212 against the infestation of semilooper and yellow mite Thus the cultivars NDC-8812 and NDC-9801 may be categorized as moderately resistant against semilooper and yellow mite with an average infestation of 11 07 & 12.62 and 14.42 & 18.78 %, respectively.

Table 2– Performance of *capsularis* jute cultivars against semilooper and yellow mite during *kharif* 2000-02.

Cultivars	Incidence of semilooper (%)		Average (%)	Incidence of yellow mite (%)
	2000-01	2001-02		2000-01
NDC-9305	10 06 (3 21)	31 36 (5 59)	20.71	17 23 (4 15)
NDC-8812	8.63 (2.97)	13 52 (3 72)	11.07	14 42 (3 83)
NDC-9101	14 44 (3 82)	28 52 (5 35)	21 48	25 34 (5.07)
NDC-9102	11 49 (3 46)	29 90 (5.51)	20 69	20.86 (4 62)
NDC-9103	16.18 (4.04)	25 86 (5 12)	21.02	21.88 (4 68)
NDC-9801	9.56 (3 1)	15.69 (4.01)	12 62	18 78 (4 37)
NDC-9802	15.52 (3.971)	16.72 (4.13)	16.12	25 90 (5 05)
NDC-9901	26.36 (5.17)	21.19 (4.64)	23.77	37.73 (6.16)

Table 2 Contd

Table 2 Contd

NDC-9902	24 60 (4 99)	24 88 (5 01)	24 74	31 29 (5 62)
NDC-9903	17 97 (4 29)	18 90 (4 38)	18 43	26 08 (5 15)
NDC-9904	21 69 (4 66)	17 05 (4 19)	19.37	25 95 (5 13)
NDC-9905	23 52 (4 87)	22 74 (4 78)	23 13	19.10 (4.42)
NDC-9906	14 75 (3 89)	15 57 (3 98)	15.16	35 10 (5 91)
JRC-212	9.58 (3 17)	16 50 (4 12)	13 04	16 48 (4.10)
JRC-321	13 11 (3 65)	25 62 (5 05)	19.36	22 29 (4 72)
UPC-94	21 64 (4 65)	21 74 (4 69)	21 69	29 88 (5 43)
CD (5%)	0 76	0 70	-	0 89
cv (%)	14 14	11 29	-	13 66

-Figures in parentheses are transformed values

-Incidence of yellow mite was negligible during *kharif* 2001-02

The overall performance of thirteen promising *capsularis* jute cultivars and three standard jute varieties revealed that the *capsularis* jute cultivars NDC-8812 & NDC-9801 are resistant to stem weevil and moderately resistant to semilooper and yellow mite.

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